The American Journal of Medicine

The American Journal of Medicine

You can prevent attacks in angina pectoris

Effective protection

Of all the drugs tested by Russek and co-workers[1] only Peritrate was found to exert a prolonged prophylactic effect in angina pectoris. In fact, their results in angina pectoris patients receiving Peritrate "... were comparable to those obtained with glyceryl trinitrate [nitroglycerin], but the duration of action was considerably more prolonged."

Simple regimen

Together with significant improvement in the EKG,[1,2] Peritrate prophylaxis will reduce the nitroglycerin need in most angina pectoris patients.[3] A continuing schedule of only 1 or 2 tablets 4 times daily will usually

1. *reduce the number of attacks in almost 80 per cent of patients*[2,3]
2. *reduce the severity of attacks which cannot be prevented.*

Available in 10 mg. tablets in bottles of 100, 500 and 5000.

1. Russek, H. I.; Urbach, K. F.; Doerner, A. A., and Zohman, B. L.: J.A.M.A. *153*:207 (Sept. 19) 1953.
2. Humphreys, P., *et al.*: Angiology 3:1 (Feb.) 1952.
3. Plotz, M.: New York State J. Med. *52*:2012 (Aug. 15) 1952.

Peritrate®

TETRANITRATE

(BRAND OF PENTAERYTHRITOL TETRANITRATE)

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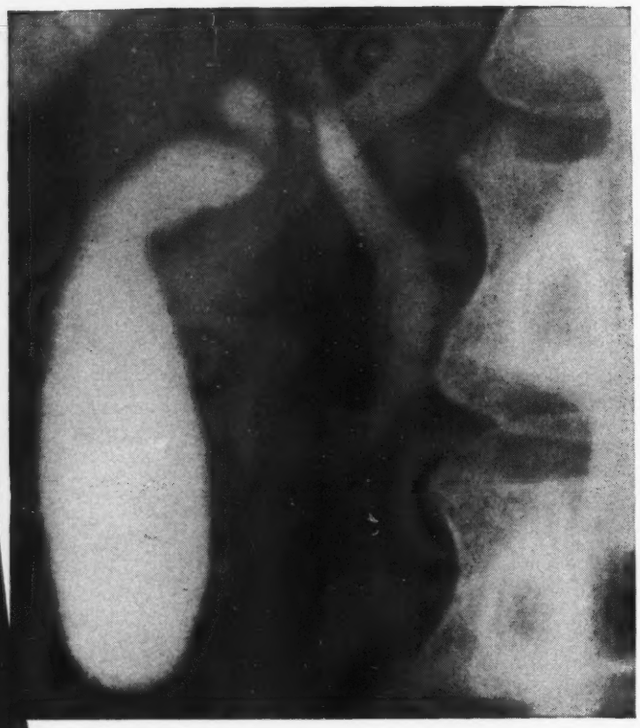
Laboratories NEW YORK



"A superior medium for oral cholecystography ... giving new and more exact diagnoses of biliary abnormalities."[1]

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Telepaque®

Superior Oral Cholecystographic AND CHOLANGIOGRAPHIC Medium

The frequency of bile duct visualization with Telepaque plus the high incidence of dense gallbladder shadow[2] are advantages of distinct diagnostic importance. Furthermore, Telepaque is notable for its low degree and percentage of side reactions.

DOSAGE: The average adult dose of Telepaque is 6 tablets with at least one glass of water from ten to twelve hours before the scheduled roentgen examination.

SUPPLIED in tablets of 0.5 Gm., envelopes of 6 tablets, boxes of 5 and 25 envelopes; bottles of 500 tablets.

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CONTENTS

The American Journal of Medicine

Vol. XVI MARCH, 1954 No. 3

Editorial

Clinical Studies

In the important matter of excluding pheochromocytoma as the remediable cause of paroxysmal or sustained hypertension, we have been largely dependent hitherto upon indirect evidence procured by observing the effects of various adrenolytic or sympatholytic agents upon the blood pressure. A more reliable indication of excessive secretion of pressor catecholamines would be their direct measurement in blood or urine. Suitable methods, however, have been lacking and development of practical procedures for this purpose has proved to be a formidable problem. Dr. Goldenberg and his collaborators have addressed themselves to this problem and the results of their long, painstaking investigations are incorporated in this article. They have succeeded in working out both a relatively simple chemical screening test and a more laborious confirmatory test for detection of excessive excretion of nor-epinephrine and epinephrine in the urine—a notable advance in diagnostic methods for pheochromocytoma.

The authors have made an elaborate study directed toward appraising the functional capacity of the adrenals more precisely than has yet been possible. They used a twofold test: a low sodium diet to provoke evidences of acute adrenal insufficiency, and intravenous corticotrophin to measure cortical responsiveness. Nine patients who had undergone subtotal adrenalectomy for hypertension and six healthy controls served as subjects for evaluation. The relevancy of the results to the known state of the adrenals, and the consistency of the several criteria employed, encourage confidence in this method of evaluation which is, however, not without risk.

The differentiation of hyperplasia from neoplasia of the adrenal cortex has important clinical implications and the evidence here adduced for usefulness of the "cortisone test" in this connection

Contents continued on page 5

CONTENTS

The American Journal of Medicine

Vol. XVI MARCH, 1954 No. 3

Contents continued from page 3

Contents continued on page 7

CONTENTS

The American Journal of Medicine

Vol. XVI MARCH, 1954 No. 3

Contents continued from page 5

Contents continued on page 9

CONTENTS

The American Journal of Medicine

Vol. XVI MARCH, 1954 No. 3

Contents continued from page 7

Seminars on Liver Disease

This is a timely review of the mechanisms of ascites formation in Laennec's cirrhosis and congestive cirrhosis, bringing together in constructive integration the results of recent experimental and clinical observations. The authors express a changing point of view in this connection. For example, the importance of increase in pressure in the hepatic veins, as opposed to the portal system, is stressed in pathogenesis, as is the role of sodium retention in addition to hypoalbuminemia. The origin of ascitic fluid is reconsidered, with special emphasis upon its exudation from the liver itself, due to augmented liver lymph flow. The implications as to management are indicated. Altogether, an informative and thought-provoking essay well worth careful study.

Clinico-pathologic Conference

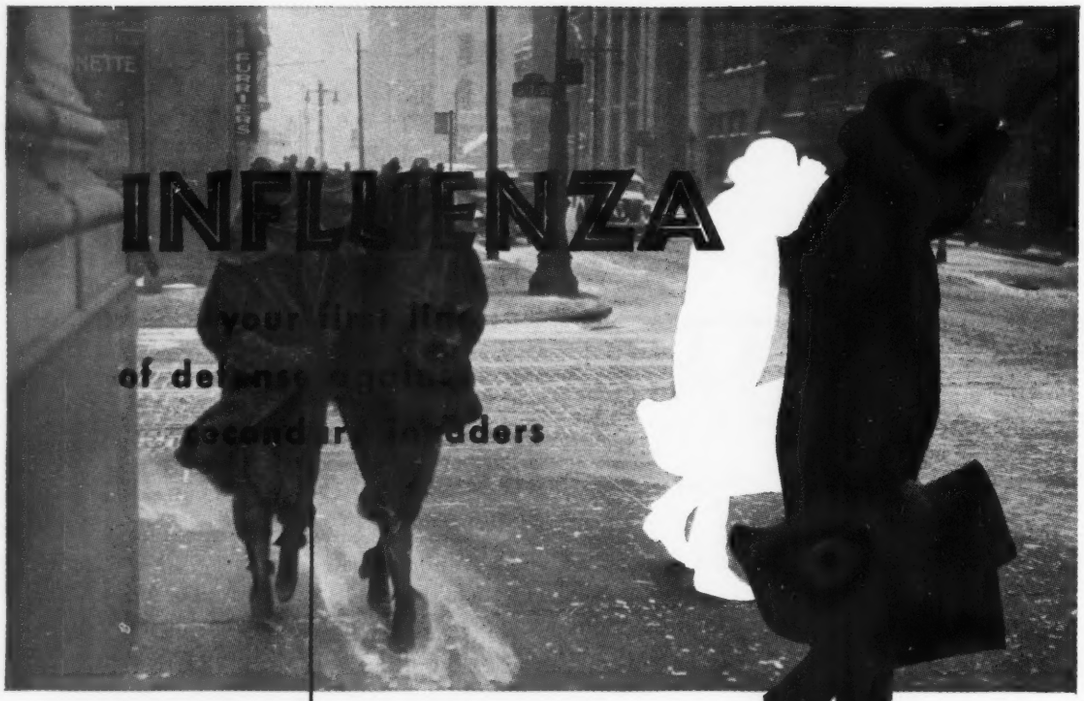
Clinico-pathologic Conference (Washington University School of Medicine)—The interest in the problem in differential diagnosis of this patient lies in the frequency with which it occurs in ordinary medical experience. The case also illustrates how difficult decision as to the presence of metastatic carcinoma may be, and how elusive location of the primary site.

Case Report

An instructive account of a case of portal hypertension, with fatal rupture of esophageal varices, due to congenital malformations of the portal venous system demonstrated at necropsy.

Advertising Index on 3rd Cover

Change of address must reach us one month preceding month of issue.



The synergistic action of Deltamide w/Penicillin promotes faster patient recovery in prevention and control of infections secondary to influenza. In addition, Deltamide w/Penicillin produces effective and well-sustained blood levels.

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Deltamide w/Penicillin is available in two dosage forms—tablets and pleasant-tasting, chocolate-flavored suspension.

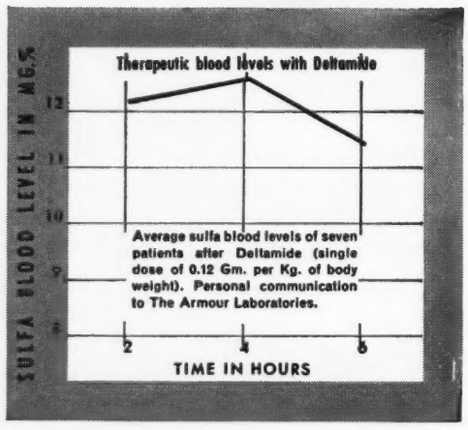
THE NEW QUADRI-SULFA MIXTURES

Each tablet, or each teaspoonful (5 cc.), provides:

Sulfadiazine	0.167 Gm.
Sulfamerazine	0.167 Gm.
Sulfamethazine	0.056 Gm.
Sulfacetamide	0.111 Gm.
Potassium Penicillin G (Buffered)	250,000 units

Tablets: Bottles of 36 and 100.

Powder for Suspension: 60 cc. bottles to provide 2 oz. of suspension by the addition of 40 cc. of water. Deltamide and Deltamide w/Penicillin Suspensions are preferred because of extreme palatability.



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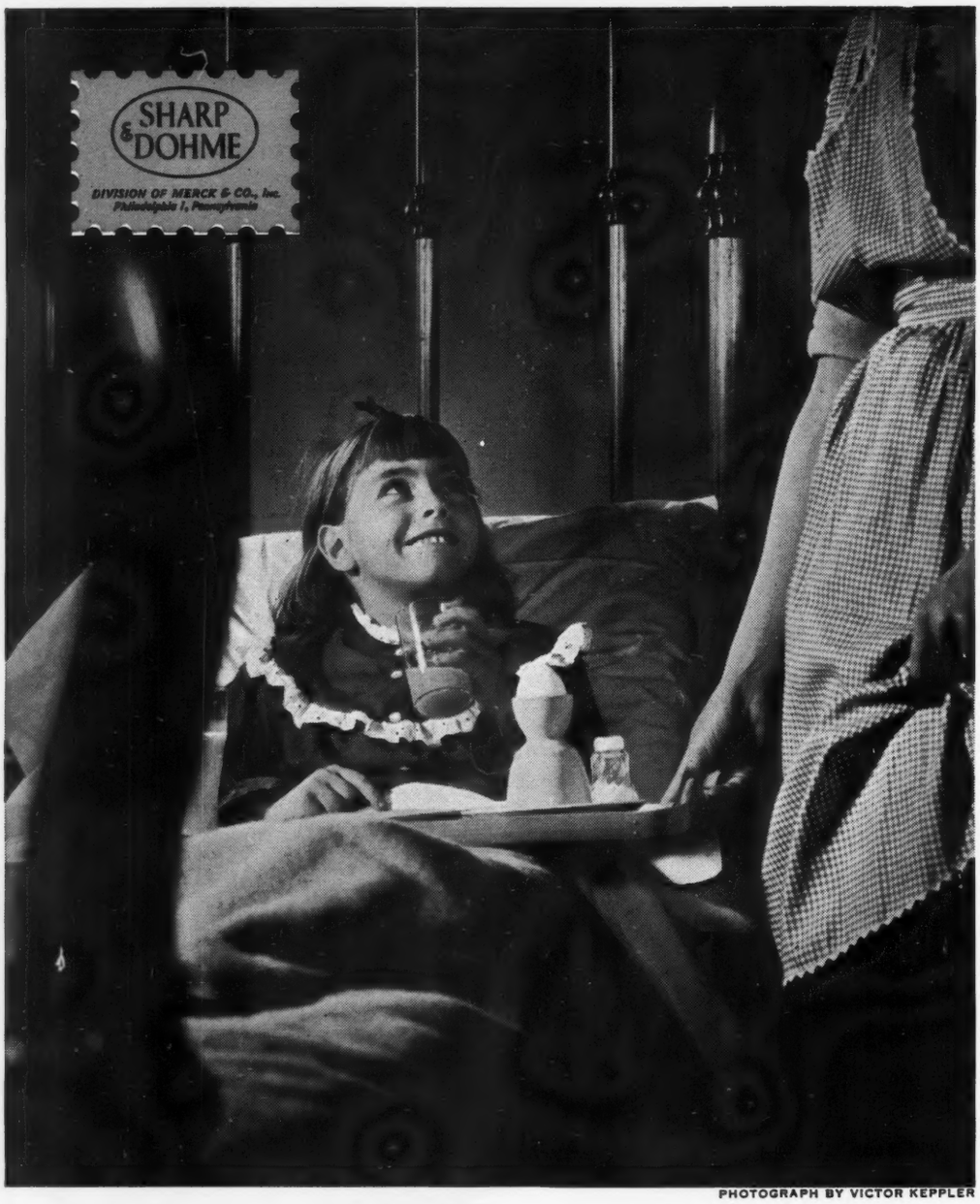

PHOTOGRAPH BY VICTOR KEPPLER

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BACITRACIN-TYROTHRICIN TROCHES WITH BENZOCAINE

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Two topical antibiotics combined, bacitracin and tyrothricin, give your patients double antibacterial action, while benzocaine provides a soothing, anesthetic effect. In severe throat infections, TRACINETS conveniently supplement systemic therapy.

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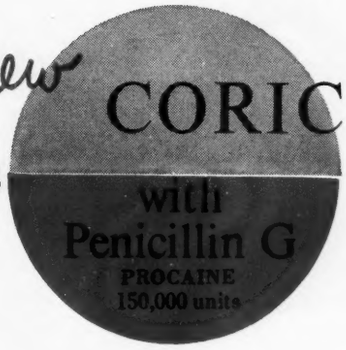
CORICIDIN

controls colds...curbs complications

...complicated colds



combats secondary invaders
antibiotic + antihistaminic, analgesic, antipyretic

...simple colds

most widely prescribed preparation for prevention and treatment of symptoms

...and for pain

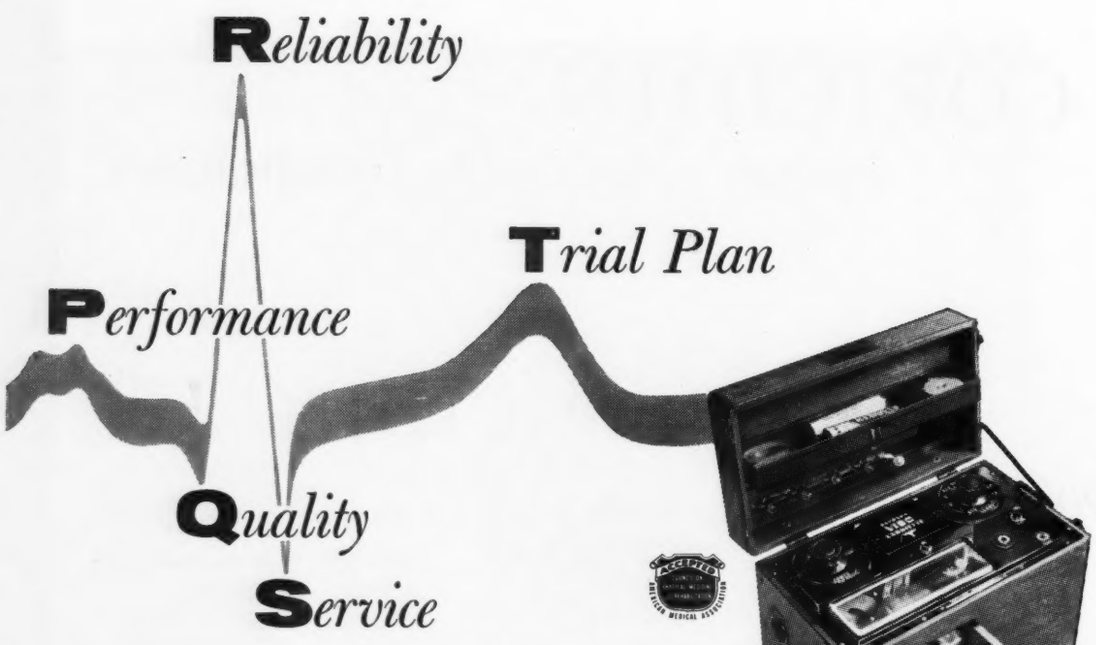
valuable in sinusitis, headache, myalgia, neuralgia, pleurisy, bursitis, grippe

*Subject to Federal Narcotic Regulations.

Each CORICIDIN® Tablet contains CHLOR-*TRIMETON*® Maleate, aspirin, acetophenetidin, and caffeine.

Schering

CORICIDIN



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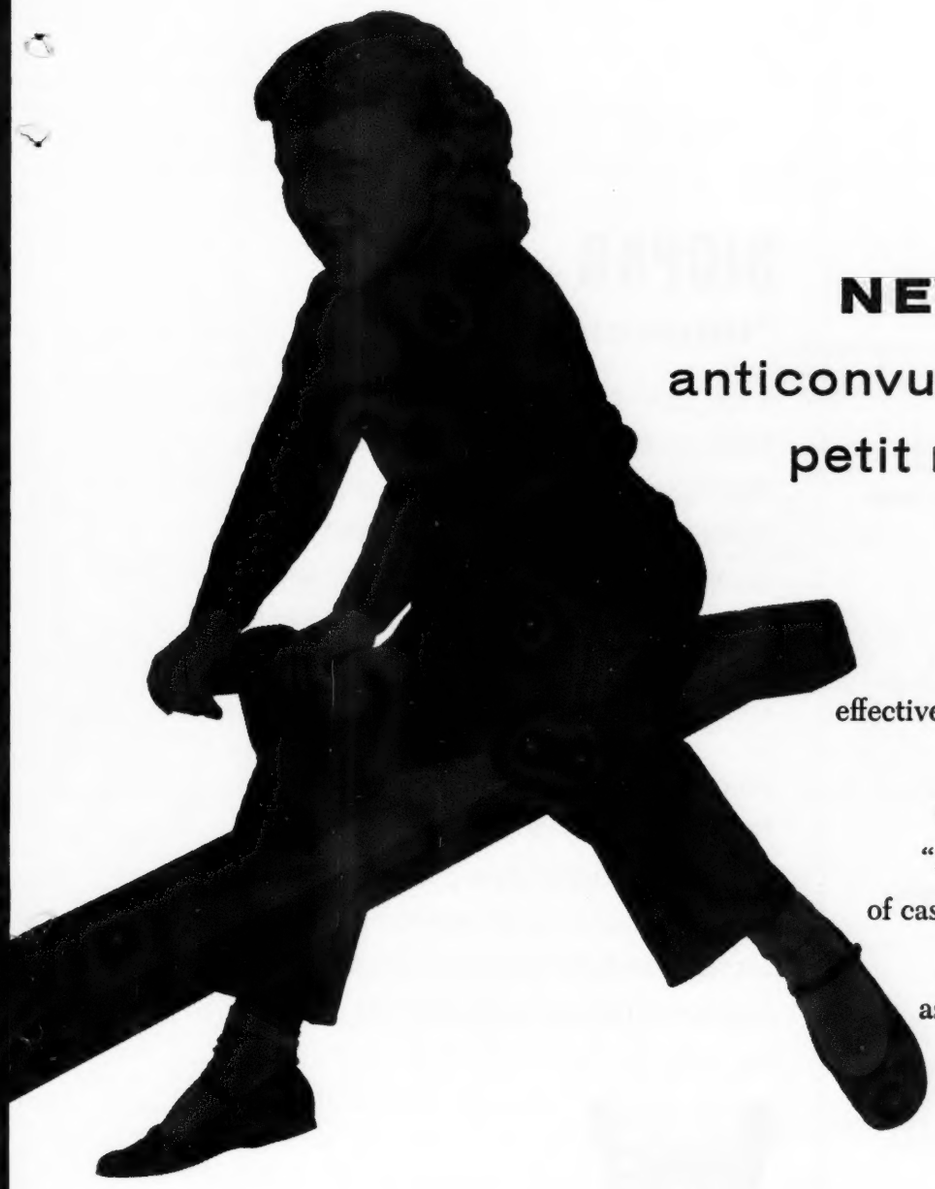

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anticonvulsant for petit mal epilepsy

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"...more efficacious in that group of cases in which standard medication gave only indifferent-to-fair results, as well as in those cases having the lowest frequency of pretreatment seizures."*

*Zimmerman, F. T.:
Am. J. Psychiat. 109:767, 1953.

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MILONTIN, a drug of choice for petit mal epilepsy, was developed by the research laboratories of Parke, Davis & Company following 11 years of study and clinical investigation.
It is available in 0.5 Gm. Kapseals in bottles of 100 and 1000.

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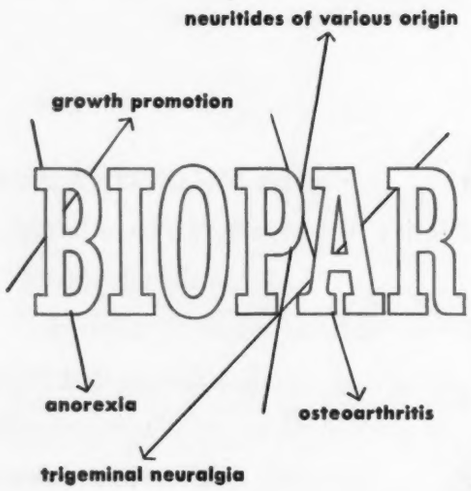

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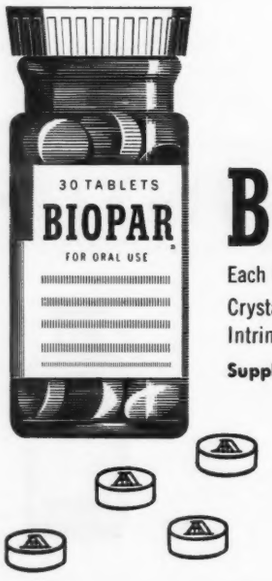

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Crystalline Vitamin B_{12} U.S.P.... 6 mcg.
Intrinsic Factor... .....30 mg.
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...for wide-spectrum antibacterial therapy: GANTRICILLIN®-300 'Roche.' Each tablet provides 300,000 units of penicillin PLUS 0.5 Gm of Gantrisin®, the single, highly soluble sulfonamide.

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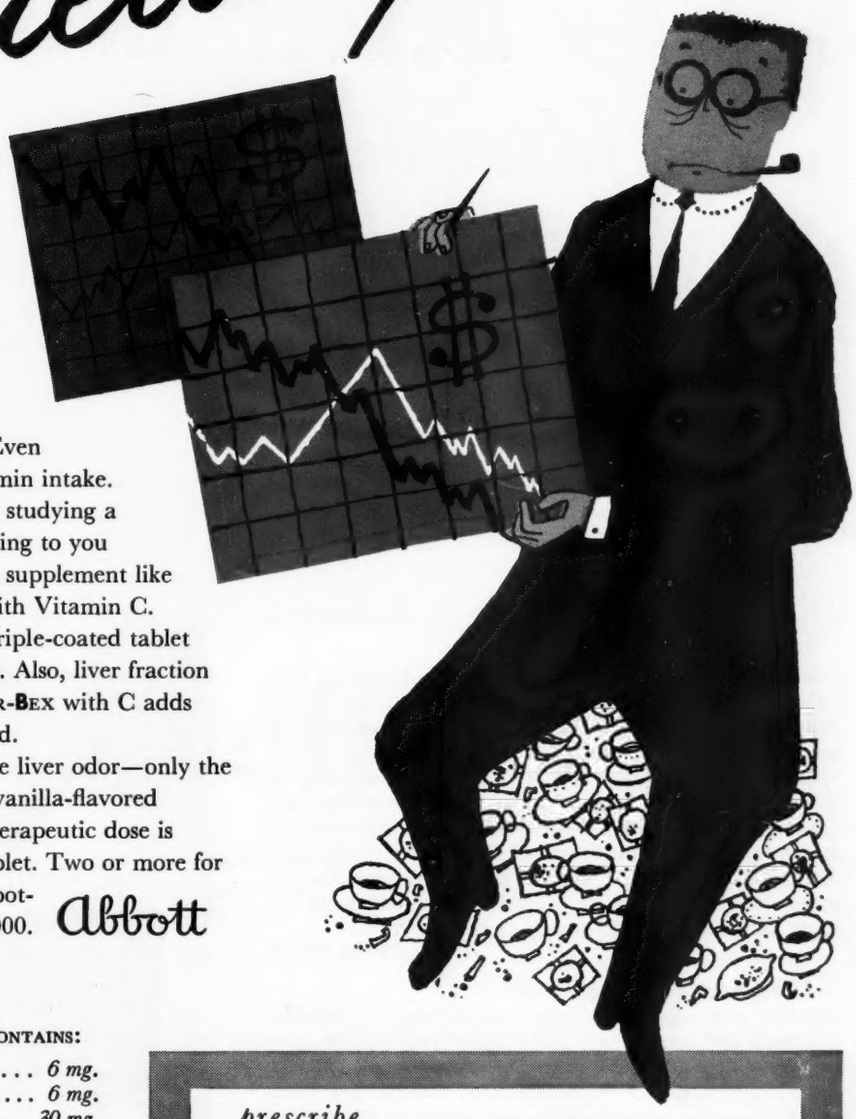

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Pantothenic acid (as calcium pantothenate)	*10 mg.*
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Brewer's yeast, dried	*0.15 Gm. (2½ grs.)*

SUR-BEX WITH VITAMIN C contains 150 mg. of ascorbic acid in addition to the vitamin B complex factors.

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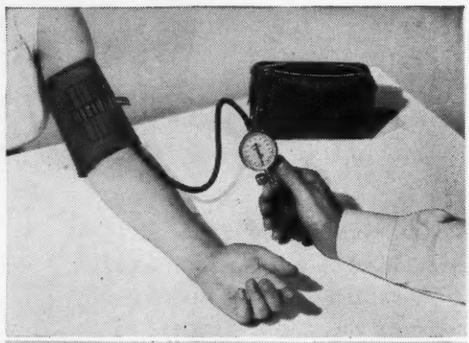
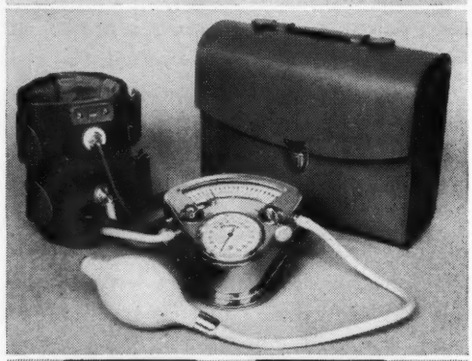
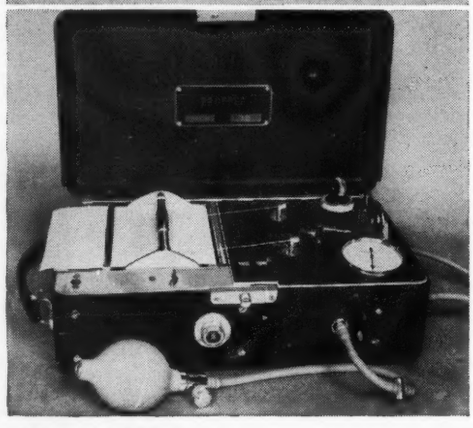
For the physician who desires the ultimate in vascular diagnosis and case history. The Propper oscillograph gives *simultaneous* pulse readings of two extremities—and provides a permanent, written record of the test. For comparative studies and complete case histories the oscillograph is as important as the EKG. The most complete equipment—in one handy case—available only from Propper. For full information, write to:







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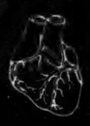
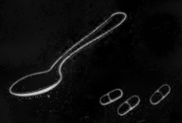
condition	 *incidence of liver dysfunction*	 *incidence of blood lipid abnormalities*	 *suggested therapy*
obesity	frequent + + +	frequent + +	Methischol plus balanced low calorie diet.
diabetes	frequent + + + +	frequent + + +	Methischol as adjunct to diet. Insulin as necessary.
atherosclerosis	frequent + + +	frequent + + + +	Methischol and high protein, low fat diet.
coronary disease	frequent + + +	frequent + + + +	Methischol as adjunct to high protein, low fat diet and specific therapy.
alcoholism	frequent + + +	frequent + +	Methischol plus high protein diet.

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... because it provides vitamin B_{12} and liver fractions in addition to choline, methionine and inositol.

... helps normalize liver function, increase phospholipid turnover, reduce fatty deposits, and stimulate regeneration of new liver cells ...

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now— higher B_{12}

the suggested daily therapeutic dose of 9 capsules or 3 tablespoonfuls of Methischol provides:

Choline Dihydrogen Citrate*	2.5 Gm.
dl, Methionine	1.0 Gm.
Inositol	0.75 Gm.
Vitamin B_{12}	18 mcg.
Liver Concentrate and Desiccated Liver**	0.78 Gm.

*Present in syrup as 1.15 Gm. Choline Chloride
**Present in syrup as 1.2 Gm. Liver Concentrate

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estrogen-progesterone
effective in
menstrual disturbances:

Cyclogesterin
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Estrogenic Substances* .. 1 mg.
(10,000 I.U.)

Progesterone .....30 mg.

**Naturally-occurring equine estrogens (consisting primarily of estrone, with small amounts of equilin and equilenin, and possible traces of estradiol) physiologically equivalent to 1 mg. of estrone.*

Available in bottles of 15 tablets.

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Tridione®
(Trimethadione, Abbott)

First successful synthetic agent—now agent of choice—for the symptomatic control of *petit mal*, myoclonic jerks and akinetic seizures.

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A new drug of low toxicity for *grand mal*, *petit mal*, myoclonic and mixed seizures. Effective in conditions symptomatic of organic brain damage.

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Homologue to TRIDIONE. An alternate preparation which is often effective in cases refractory to TRIDIONE therapy. For treatment of the *petit mal* triad.

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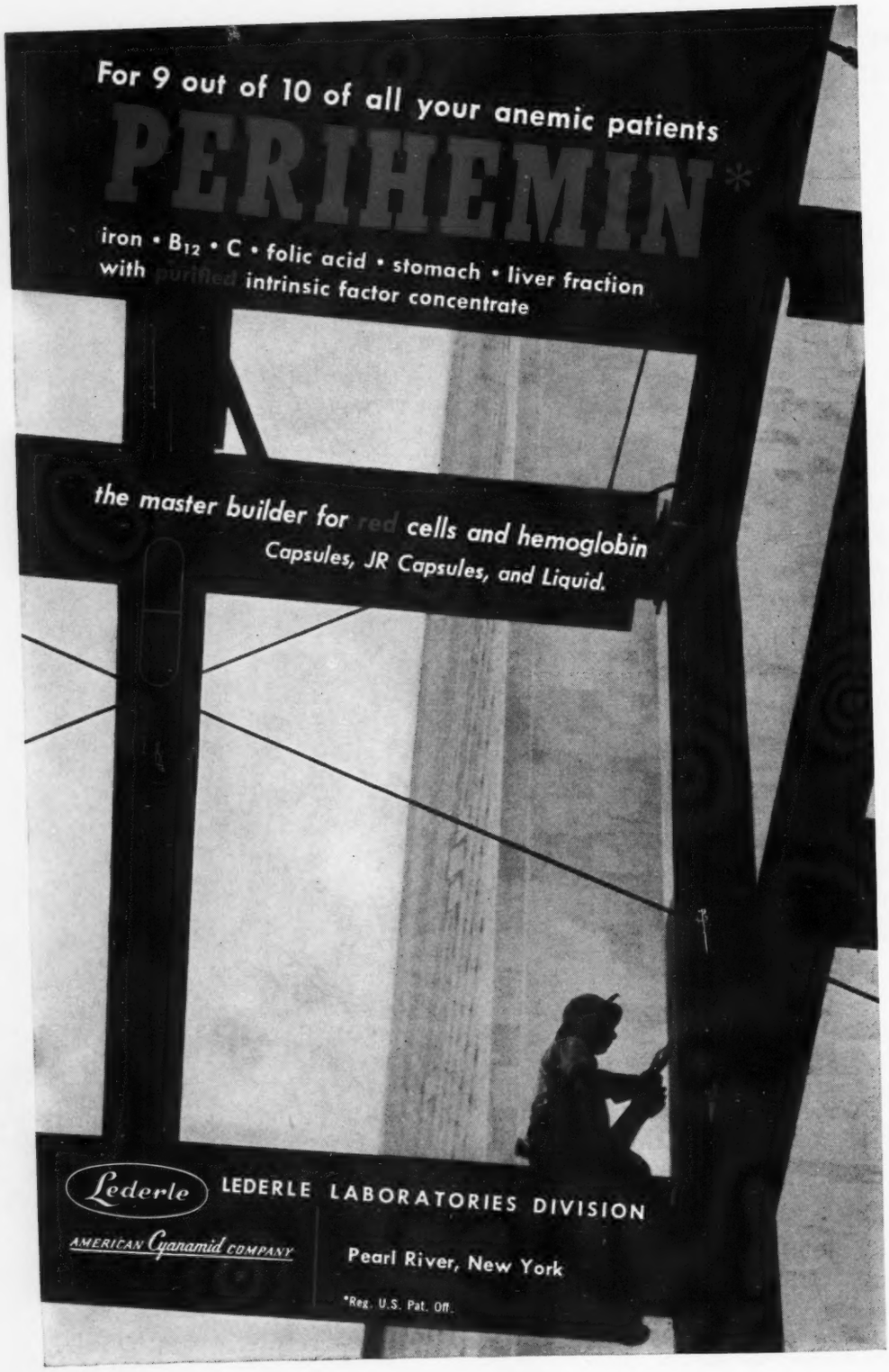
. . . for INDIVIDUALIZED anticonvulsant therapy

In epilepsy, the highly individual phenomena and their response to treatment indicate the need for *individualized* treatment. Help in accomplishing this is offered by these four modern anticonvulsants. Each is a dramatic advance in anti-epileptic medicine. Each has specific uses, advantages. With them, you may add inestimably to the scope and progress of your treatment by fitting the drug to the seizure. Remember these names. Use them wisely, carefully. But *first*—please—won't you write for literature? Abbott Laboratories, North Chicago, Ill. Abbott

1-209A


For 9 out of 10 of all your anemic patients
PERIHEMIN*
iron • B12 • C • folic acid • stomach • liver fraction
with purified intrinsic factor concentrate
the master builder for red cells and hemoglobin
Capsules, JR Capsules, and Liquid.
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"for the pain of most patients"

PHENAPHEN
the basic non-narcotic formula

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with codeine phosphate ¼ gr.

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with codeine phosphate ½ gr.

Each capsule contains: acetylsalicylic acid 162 mg. (2½ gr.), phenacetin 194 mg. (3 gr.), phenobarbital 16.2 mg. (¼ gr.), hyoscyamine sulfate 0.031 mg., codeine phosphate 16.2 mg. (¼ gr.) or 32.4 mg. (½ gr.).

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when "life situations" lead to
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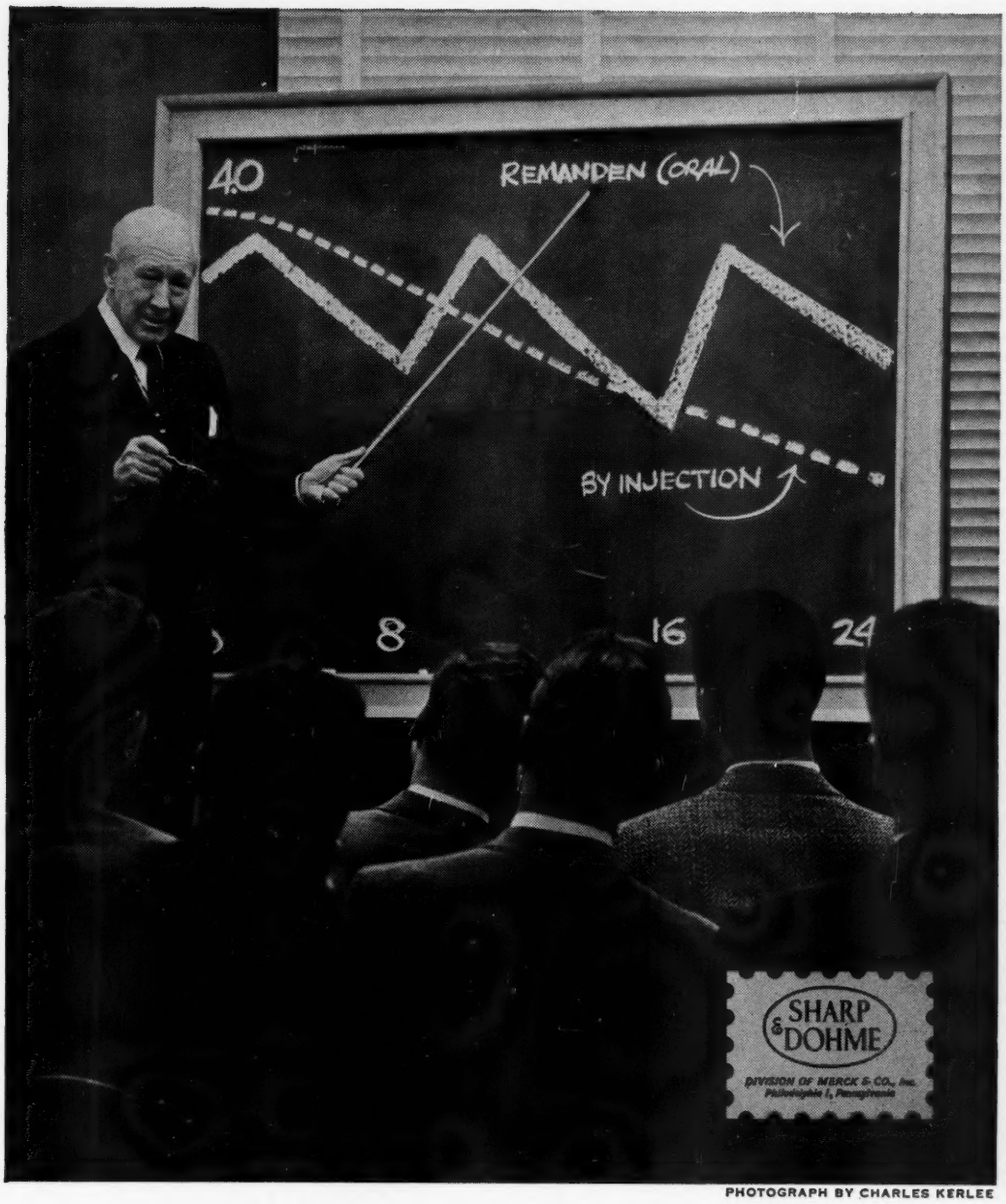
DONNATAL® PLUS
(DONNATAL WITH B COMPLEX) TABLETS ELIXIR
Robins
provides anticholinergic blocking action, mild sedation, and high level B Complex vitamin intake
Each tablet or each 5 cc. teaspoonful contains:
Hyoscyamine sulfate ..... 0.1037 mg.
Atropine sulfate ..... 0.0194 mg.
Hyoscine hydrobromide ..... 0.0065 mg.
Phenobarbital (¼ gr.) ..... 16.2 mg.
Thiamine ..... 3.0 mg.
Riboflavin ..... 2.0 mg.
Nicotinamide ..... 10.0 mg.
Pantothenic acid ..... 2.0 mg.
Pyridoxine hydrochloride ..... 0.5 mg.
A. H. ROBINS CO., INC.
RICHMOND 20, VIRGINIA



PHOTOGRAPH BY CHARLES KERLEE

Note the sustained penicillin levels with oral

REMANDEN®

PENICILLIN WITH PROBENECID

The probenecid in this oral tablet produces sustained plasma levels comparing favorably with those obtained by intramuscular procaine penicillin.[1] Compared with other oral penicillin preparations, penicillin plasma levels are 2 to 10 times higher.

Quick Information: REMANDEN-100 and REMANDEN-250 supply 0.25 *Gm.* BENEMID® (probenecid) per tablet and 100,000 or 250,000 units of crystalline penicillin G. *Dosage: Adults,* 4 tablets initially, then 2 every 6 or 8 hours. *Children,* usually 2 to 4 tablets daily.

Reference: 1. Antibiotics & Chemotherapy 2:555, 1952.

IN ECLAMPSIA

UNITENSEN®

Brand of Cryptenamine (Irwin, Neisler)

the first and only isolated Veratrum alkaloid preparation with an unparalleled safety ratio of ⟶

Unitensen provides **CRYPTENAMINE**, the first—and only—isolated Veratrum alkaloid preparation which does not induce vomiting on the same (or approximate) dose as that which exerts hypotensive action.

INDICATIONS

Eclampsia, preeclampsia, and preeclampsia with underlying essential hypertension.

Available at present as a parenteral preparation, containing 2 mg. per cc. (260 C.S.R.* Units) of cryptenamine, in 5 cc. multiple dose vials.

*Carotid Sinus Reflex

*Assayed Biologically**

Information concerning technique of administration and abstract on request.

Comparison of Unitensen with Protoveratrine and three other alkaloid preparations

DRUG	EMETIC : THERAPEUTIC RATIO	THERAPEUTIC INDEX
UNITENSEN (cryptenamine, Irwin-Neisler)	**4:1**	125
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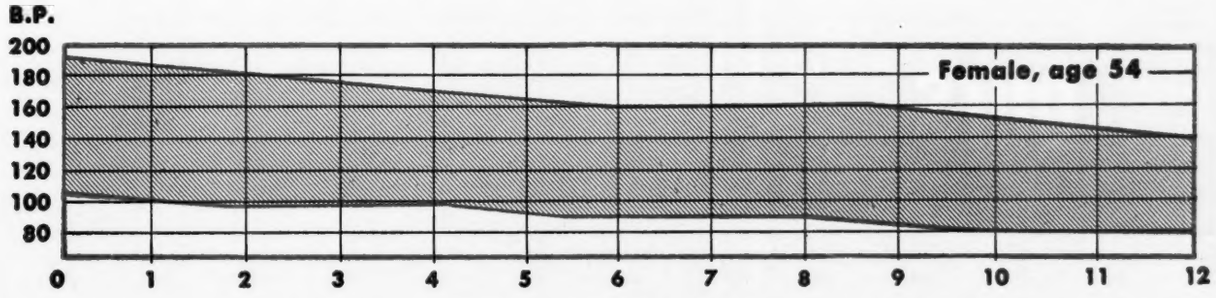

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The American Journal of Medicine

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Editorial

The Nature of the Engine

IN an earlier contribution to these pages[1] it was pointed out that the constancy of composition of the adult animal was in large part the consequence of a dynamic situation referred to as the "dynamic steady state." The dynamic steady state was contrasted with thermodynamic equilibrium and with kinetic stability, which are the usual explanations of constancy of composition in non-living systems. It results from a nice balance between the rates of synthetic, in general, energy-consuming processes and degradative, usually energy-yielding reactions. The continued operation in the living organism of energy-consuming (endergonic) processes was noted to require the operation of an engine. It is the purpose of the present contribution to examine the nature of the engine which, in biologic systems, performs the work necessary for maintenance of the dynamic steady state.

An engine, for present purposes, may be defined as a device which transmits energy derived from some source to perform some useful function. Among the numerous bodily functions which depend upon the operation of such an engine are synthesis of large molecules of protein, polysaccharide or lipid, the performance of muscle work, the absorption or secretion of solutes against concentration gradients and the initiation and transmission of nerve impulses. It appears today that it is one and the same engine which delivers the energy for these diverse processes.

The key to this engine lies in the peculiar combination of properties of certain compounds of phosphoric acid, of which pyrophosphates such as adenosine triphosphate are exemplary. The hydrolysis of pyrophosphate bonds yields relatively large amounts of energy (10,000–15,000 cal. per mol) and at the same time these compounds have sufficient kinetic stability under biologic conditions so that the expected spontaneous irreversible hydrolysis is quite slow. This happy combination of properties is found not only in pyrophosphates but also in substituted phosphamates like phosphocreatine, in enol esters like phosphopyruvic acid and in thioesters like acetyl-coenzyme A, and permits the appreciable accumulation of chemical energy in such compounds as adenosine triphosphate and phosphocreatine.

In the course of numerous catabolic processes inorganic phosphate is consumed. Certain of these phosphorylations involve the immediate reaction with phosphate of the substrate catabolized and are said to be "at a substrate level." Others are not coupled directly to the substrate but rather to the successive transport of hydrogen atoms or electrons through the series of respiratory enzymes which intervene between substrate and oxygen. This latter type of phosphorylation is termed "oxidative phosphorylation." Both in the course of anaerobic glycolysis, the non-oxidative conversion of glucose to lactic acid, and in the subsequent oxidation of products of glycolysis to CO_2, phosphorylations occur involving the consumption of inorganic phosphate, P_i, and the formation of adenosine triphosphate and related energy rich compounds of phosphorus, $\sim P$. Inherent in this scheme is the probable explanation of the so-called Pasteur effect, an important chemoregulator which provides that the more extensively oxidative reactions are proceeding, the less glucose will undergo glycolysis. An explanation of the Pasteur effect currently favored relates to the fact that the glycolytic and the oxidative (respiratory) reactions compete for available intracellular inorganic phosphate, and that in the presence of abundant oxidation a paucity of P_i limits the rate of glycolysis.

It should be stressed that at least in the case of oxidative phosphorylation the phenomenon of

[1] STETTEN, D., JR. Thermodynamic, kinetic and biologic stability. *Am. J. Med.*, 13: 251, 1952.

phosphorylation is but loosely coupled to the occurrence of oxidative reactions. Some degree of histologic integrity appears necessary for this coupling to be demonstrable. In mitochondrial suspensions consumption of P_i and formation of ~P may readily be observed to accompany the oxidation of various substrates whereas at a lower level of organization, such as in soluble enzyme systems, oxidation of the same substrates is not accompanied by phosphorylation. Various chemical agents, notably 2,4-dinitrophenol and other phenols are known to "uncouple" phosphorylation from oxidative reactions and there is reason to suspect that thyroxine may share in this uncoupling effect. Under these circumstances a tissue escapes from the regulatory inhibition of the Pasteur effect and glycolysis as well as respiration are no longer restricted by paucity of P_i. The symptoms of intoxication due to dinitrophenol and possibly also those due to excess thyroid hormone may find their explanation in this escape.

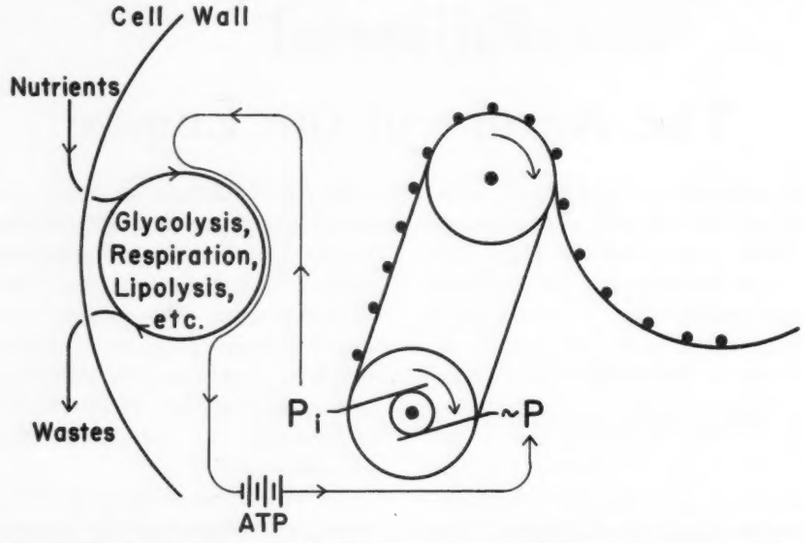

FIG. 1. The relationships of catabolic processes, coupled phosphorylations and the dynamic steady state.

It is at the expense of the energy accumulated as ~P that the several endergonic processes in the body operate. On the left side of Figure 1 is diagrammed the relationship between the major exergonic catabolic processes, preliminary breakdown of sugars and fats and the terminal oxidation of derived products, coupled with the process of phosphorylation. This method of presentation is borrowed from that of Lipmann[2] who was among the first to grasp the significance of this coupling. The right side of Figure 1 represents a dynamic steady state, indicated previously[1] as an energy summit to which balls are hauled by the operation of an engine, and from which they roll spontaneously. The engine runs from the potential difference between ~P and P_i. In conformity with Lipmann's suggestion an electrical analogy has been employed wherein adenosine triphosphate serves as a storage battery, charged by the operation of a variety of catabolic processes and discharging in so far as it drives the motor of the biochemical ski-tow.

It has been calculated that a maximum of about 70 per cent of the energy released in such processes as glycolysis or pyruvate oxidation is recoverable in the form of ~P. This estimate is a maximum, however, and is attained only if coupling is perfect. In view of the ease with which uncoupling is effected experimentally, it seems possible that the efficiency of coupling in the intact animal is less than perfect. High net efficiency is also contingent upon the absence of appreciable spontaneous or enzyme-catalyzed hydrolysis of energy rich phosphorus compounds, and such reactions of course occur. In the present analogy, such reactions might be compared with electrical leaks which wastefully drain the storage battery.

That ~P does indeed serve as the energy source for a host of endergonic processes has been clearly demonstrated in many cases and indicated in others. The biosynthesis of polysaccharides from glucose, of polypeptides from

[2] LIPMANN, F. Metabolic generation and utilization of phosphate bond energy. *Adv. in Enzymol.*, 1: 99, 1941.

amino acids, of fatty acids from acetic acid have been shown to depend upon a source of adenosine triphosphate. The performance of work by striated muscle, the transport of certain ions and molecules against gradients, and even the flashing of light by fire-flies appear to be ATP-dependent processes.

The development of biochemical thought has been so rapid that it is unwise to propose a final scheme to account for any biochemical phenomenon. A present reading of the literature suggests, however, that in the generation and utilization of high energy phosphate compounds may be the answer to the question, "What makes Sammy run." It may also resolve the quandary in which the Red Queen found herself when she discovered that she had to run faster in order to remain in the same place.

DeWitt Stetten, Jr., M.D., Ph.D.

Clinical Studies

Chemical Screening Methods for the Diagnosis of Pheochromocytoma*

I. Nor-epinephrine and Epinephrine in Human Urine

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New York, New York

HYPERFUNCTION of the adrenal medulla in man is due to the presence of chromaffin tissue tumors, pheochromocytomas, which may originate in the adrenal medulla or wherever chromaffin tissue is found in the body during early life. Such pheochromocytomas give rise to different clinical syndromes which can be characterized as follows: (1) Paroxysmal hypertension (adrenal sympathetic syndrome); (2) persistent hypertension mimicking essential or malignant hypertension; (3) a combination of hypertension, hypermetabolism and glycosuria and (4) persistent hypermetabolism or hyperglycemia coexistent with intermittent hypertension.

Better insight into the pathogenesis of these widely varying syndromes has been gained by the demonstration that these tumors elaborate not one but two distinct agents, epinephrine and nor-epinephrine, in varying proportions.[1,2] Contributing to the variation in clinical syndromes are such additional factors as the rate of secretion of the tumors and secondary endocrine and vascular (smooth muscle) changes.

If tumors of the adrenal medulla functioned in the manner of the normal medulla, resting secretion would be negligible and a discharge of epinephrine and nor-epinephrine would occur only upon physiologic stimulation, e.g., by acetylcholine or histamine. This is true, in part, for a fraction of the cases studied, one-third according to Green[3] and one-fourth in our own series,[4] which represent the classic type of pheochromocytoma. The resulting clinical picture is characterized by paroxysms (sometimes termed adrenal sympathetic syndrome) which are comparable in their manifestations to the effects of a rapid intravenous injection of a pharmacologic dose of epinephrine and/or nor-epinephrine. The chief features of such an attack are a steep rise in blood pressure associated with pallor, tachycardia, precordial and upper abdominal pain, hyperglycemia and anxiety, all or some of which may last for many minutes, or even hours. The attack then usually subsides but occasionally leads to fatal pulmonary edema or ventricular fibrillation. The differences in the ratio of epinephrine to nor-epinephrine in these tumors should not influence the clinical picture since the sudden discharge of large amounts of epinephrine will cause over-all vasoconstriction, just as nor-epinephrine does, while large amounts of nor-epinephrine will cause concomitant metabolic changes.[5] Observations on cases which combine paroxysmal hypertension with persistent hypermetabolism or persistent hyperglycemia[6,7] suggest that the "resting secretion" of these tumors may not be negligible although insufficient to produce changes in blood pressure. This may well be due to the fact that metabolic effects are caused by much smaller doses of epinephrine than those required to produce hypertension.[8]

More often, pheochromocytomas are asso-

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ciated with *persistent* hypertension. The adrenergic blocking effect characteristically elicitable in these cases suggests that this form of sustained hypertension is due to continuous secretion of epinephrine and/or nor-epinephrine by the tumor.[4,5] An instructive correlation between the clinical and chemical data could be made out in this group.[4,5] Small tumors which contained nor-epinephrine predominantly (90 to 97 per cent) but not more than a total of 80 mg. of this catecholamine gave rise to a syndrome mimicking essential hypertension, not accompanied by striking metabolic features. In patients with tumors containing larger total amounts of nor-epinephrine, hypermetabolism and hyperglycemia were more marked, even though nor-epinephrine causes these to a much lesser degree than an equal amount of epinephrine. When epinephrine was the predominant catecholamine in the tumor, hypertension, hypermetabolism, hyperglycemia and tachycardia were all prominent clinical manifestations.

A surprising observation was that patients with tumors containing large quantities of epinephrine may, at times, present a clinical picture indistinguishable from that of essential hypertensive vascular disease, with normal heart rate, absence of hyperglycemia, absence of metabolic disturbance as indicated by normal basal oxygen consumption, and a negative or equivocal response to benzodioxane. This suggests that persistent hypertension in patients with pheochromocytoma is not invariably due to the presence in the circulation of sufficient quantities of nor-epinephrine or epinephrine to cause hypertension by direct cardiovascular action, i.e., of the sort seen in acute infusion experiments.[4]

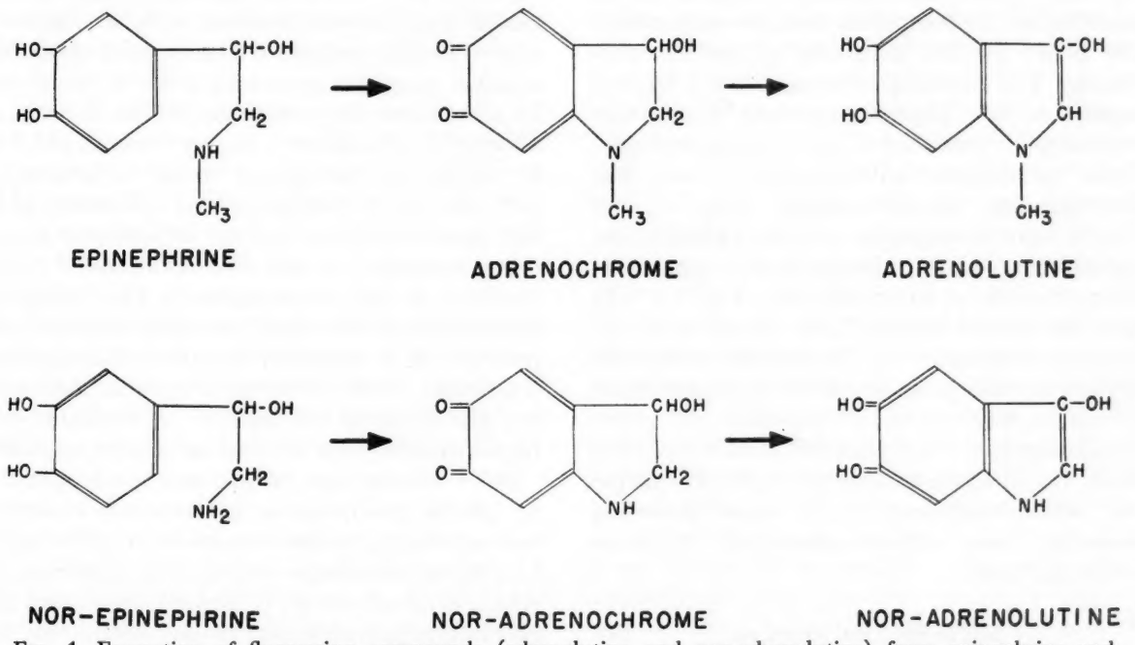

FIG. 1. Formation of fluorescing compounds (adrenolutine and nor-adrenolutine) from epinephrine and nor-epinephrine.

This last mentioned phase of "non-humoral" hypertension in pheochromocytomas in particular limits the value of adrenergic blocking agents in the diagnosis of this disease.[9–11] Although the incidence of false negative tests does not exceed 10 per cent (vide seq.), a direct diagnostic method which excludes the variability of the pharmacologic response would be preferable. The increased urinary output of nor-epinephrine and epinephrine in patients with pheochromocytoma seemed to offer the possibility of successfully developing such a method.

Urinary excretion of epinephrine and other sympathomimetic amines after their ingestion was first demonstrated by Richter in 1940.[12] The occurrence of catecholamines in normal human urine was first indicated by Holtz et al. in 1947[13] and was confirmed in 1951 by von Euler and Hellner.[14] The diagnostic applicability of the presence of increased urinary output of epinephrine and nor-epinephrine in pheochromocytoma was first described by Engel and von Euler in

1950[15] and subsequently corroborated by Goldenberg and Rapport,[16] and von Euler.[17] The method employed by these investigators necessitated the use of a bio-assay procedure after suitable urinary adsorption procedures.

We have endeavored to replace the bio-assay procedure by chemical methods suitable for the quantitation of epinephrine and nor-epinephrine and hence for the diagnosis of pheochromocytoma. The chemical methods studied depend largely on the photofluorescence of reaction products of epinephrine and nor-epinephrine. These substances, adrenolutine[18,19] and the corresponding nor-adrenolutine (Fig. 1), are formed from epinephrine and nor-epinephrine, respectively, with adrenochrome and noradrenochrome as intermediates. (Fig. 1.) The methods studied in the course of our work include the following: (1) Preparation of urinary extracts by adsorption on precipitated aluminum hydroxide, followed by (*a*) bio-assay, (*b*) paper chromatography, (*c*) photofluorometric evaluation, (*d*) absolute quantitation of epinephrine and nor-epinephrine; (2) A rapid screening procedure using column adsorption; (3) pharmacologic tests.

METHODS AND RESULTS

1. Adsorption of Catechol Amines on Precipitated Aluminum Hydroxide[14]

Collection of Urine. Twenty-four-hour urine specimens are collected with 10 ml. of 6 N HCl as a preservative for catecholamines. The pH should be approximately 3.5. The specimens are kept refrigerated. The use of metal urinals or funnels is to be avoided. The patient should, if possible, be kept off medication, particularly vitamins, in order to prevent interference with the photofluorometric tests.

Hydrolysis of Urine. Samples of 525 ml. of acidified urine are filtered and the filtrate adjusted to pH 1.5–2.0 with 6 N HCl (using short range hydrion paper), placed in a cork-stoppered, 1 L. round bottom flask and heated in a boiling water bath for fifteen minutes with occasional shaking. After slight cooling the pH is redetermined with hydrion paper. If the value exceeds 2.0, it is readjusted to pH 1.5 to 2.0. The heating is repeated for fifteen minutes in any case. The urine sample is then cooled in an ice bath to room temperature.

Adsorption on Aluminum Hydroxide. To a portion of hydrolyzed urine equivalent to 500 ml. of the original (usually about 510 ml.) 7.5 ml. of 20 per cent (w/v) aqueous aluminum sulfate solution is added. The pH is adjusted to 7.6 to 7.8 (glass electrode) by dropwise addition of 5N NaOH with vigorous continuous stirring.

It has been found necessary to use a continuous reading, line operated pH meter (Beckman model H-2) in conjunction with a magnetic stirrer for this operation. The amount of NaOH solution required is usually from 8 to 12 ml. In some cases the precipitate begins to form at about pH 4; in others it begins between pH 5 to 6; rarely, no precipitate at all is formed at pH 7 to 7.5. In this latter case it is essential to add more aluminum sulfate. In one case it was found necessary to add two additional 7.5 ml. portions to get a precipitate. The complete adsorption of the catecholamines requires the presence of a sufficient quantity of aluminum hydroxide, while too much adsorbent decreases the specificity of the method by retaining too high a urine volume in the gelatinous precipitate.

After stirring one or two minutes at pH 7.6 to 7.8 the precipitate is immediately collected by centrifuging for fifteen minutes at 1,800 r.p.m. in 250 ml. centrifuge bottles. The supernatant, which is usually clear, is then decanted and the precipitate is transferred to one bottle and resuspended in 30 ml. saline which has been adjusted to pH 7.8 (500 ml. saline + 1 ml. 0.2 M phosphate buffer, pH 7.8). This saline wash is used to aid the transfer of precipitate (saline is employed to inhibit the tendency of aluminum hydroxide to form colloidal suspensions in H_2O). The suspension is centrifuged for thirty minutes at 1,800 r.p.m. and the supernatant is discarded if it is clear or even somewhat opalescent.

A precipitate volume of about 10 to 20 ml. after the final centrifuging has been found very satisfactory. If the volume is below this, the quantity of aluminum sulfate has not been sufficient for the particular sample and it may be best to repeat the precipitation.

A very milky saline supernatant is not discarded, the washing step in effect being eliminated. It has proved better to sacrifice the specificity of the method by eliminating the washing than to accept the loss which results from discarding the colloidal suspension. This occurs with less than 5 per cent of the urine specimens.

The washed gelatinous precipitate is then completely dissolved by stirring with the mini-

mum quantity of 6N H_2SO_4 (usually 2.5 to 3.0 ml.). At this stage the material can usually be allowed to stand without much danger of destruction of catecholamines. It is convenient to set the mixture in the refrigerator overnight, since the precipitate dissolves slowly because of the gelatinous lumps. On examination after overnight refrigeration a granular precipitate is frequently present which is quite different from the original one.

The pH of the mixture is then adjusted to pH 3.5–3.9 (glass electrode—Beckman model G meter) by the dropwise addition of 5N NaOH to pH 3 and 1N NaOH from then on. Indicator paper may be used up to pH 3.0 but then the pH meter should be used. Vigorous shaking is necessary after the addition of each drop to avoid local concentration of alkali, especially after the heavy precipitate of aluminum hydroxide forms and the mixture becomes quite thick. The operation is carried out by vigorous hand shaking in a 250 ml. centrifuge bottle and using a plastic stirrer. The volume of the mixture is then estimated to the nearest 5 ml. (marking the centrifuge bottle at 10 ml. and at 5 ml. increments to 50 ml. facilitates this greatly). Four volumes of a solution of acetone—95 per cent ethanol (1:1) are then added. The mixture is shaken until uniform and then chilled at 4°C. for three hours, or overnight.

Preparation of the Final Extract. The mixture is centrifuged for thirty minutes, the supernatant solution then being carefully decanted. The residue is washed by adding 30 ml. of the 1:1 acetone–95 per cent ethanol, stirring until homogeneous and recentrifuging. The supernatant solution is combined with the first, and careful evaporation to a volume of 1 to 2 ml. is carried out in vacuo, employing a water-pump and a bath temperature not above 45°C. The bath is lowered as required to keep its level below that of the liquid in the flask. It has been found that excessive drying in the presence of acidified 95 per cent ethanol causes a loss of nor-epinephrine by ether formation.[20] The residue is removed with small portions of 1:1 acetone-alcohol and transferred to a 25 cc. pear-shaped flask. The flask is fitted with an adapter which allows a current of air to pass over the surface of the liquid when suction is applied to a side-arm. The solution is then reconcentrated in vacuo at 45°C. and again the bath is lowered so that excessive drying does not occur on the sides. The residue is transferred to a 5 ml. volumetric flask and made to volume using 0.01 N hydrochloric acid.*

1a. Bio-assay: Method: The urine extracts obtained by adsorption on aluminum hydroxide, desalting and concentration in vacuo (usually 100:1) are taken up in 0.01 N HCl and quantitated by bio-assay. Cats are anesthetized with chloralose 60 mg./kg. intraperitoneally† and the blood pressure responses recorded before and after sensitization with cocaine, 4 to 8 mg./kg. The bio-assay values are expressed as nor-epinephrine equivalents. The ratio of equipressor doses of nor-epinephrine: epinephrine varies in different experiments and changes following cocainization. One part nor-epinephrine is usually equivalent to 2 to 4 parts of epinephrine; in occasional experiments the ratio was 1:5. The nor-epinephrine equivalent, as determined, therefore sometimes understates the sum of epinephrine + nor-epinephrine excreted in urines. This error will decrease the difference between essential hypertensive and pheochromocytoma urines. In urine extracts which on paper chromatography showed high epinephrine:nor-epinephrine ratios, i.e., greater than 40 per cent, quantitation by planimetric estimation of the epinephrine and nor-epinephrine spots was attempted. This was followed by bio-assay (cat blood pressure) using epinephrine + nor-epinephrine mixtures (calculated from the results of paper chromatography) to match the blood pressure response of the urine extract.

This procedure seemed preferable to the use of differential bio-assays which are based on the quantitative difference in response to the two compounds on two different pharmacologic preparations, of which one is predominantly sensitive to epinephrine, the other one more or equally sensitive to nor-epinephrine. By the use of mathematical formulas the epinephrine and nor-epinephrine content of the mixture is then calculated. Gaddum and Lembeck[21] showed that such tests may be used to prove that a mixture of the amines is present but that they do not give a reliable estimate of their concentration. This criticism of the differential bio-assay of mixtures of epinephrine and nor-epinephrine

* If it is desired to use the extract for identification purposes by means of paper chromatography, the residue is taken up and made to volume in 95 per cent EtOH containing 1 cc. conc. HCl per 100 cc.; in the quantitative studies (cf. seq.) the residue is taken up in 5 per cent acetic acid.

† 20 mg. chloralose per ml. 70 per cent ethanol.

led to the development of a new method for the quantitative separation of epinephrine and nor-epinephrine in biological fluids by Crawford and Outschoorn.[22] Their method separates the amines first by paper chromatography and quantitates the single eluted amines by their effect on the rat's blood pressure.

TABLE I

TWENTY-FOUR-HOUR URINARY EXCRETION OF CATECHOLAMINES IN THIRTEEN NORMOTENSIVE HEALTHY SUBJECTS AND IN THIRTY-FIVE CASES OF ESSENTIAL HYPERTENSIVE VASCULAR DISEASE

Nor-epinephrine Equivalent (μg.)	No. of Cases	
	Normotensives	Essential Hypertensives
7–10	0	4
11–20	4	10
21–30	6	0
31–40	2	4
41–50	1	2
51–60	0	2
61–70	0	2
71–80	0	1
81–90	0	0
91–100	0	1

1a. Bio-assay: Results: The urinary excretion of catecholamines was studied by bio-assay in thirteen normotensive subjects, in thirty-five cases of essential hypertensive vascular disease (two of these in the malignant phase) and in sixteen cases of pheochromocytoma. Ten cases of Addison's disease and fifteen patients with essential hypertensive vascular disease who had undergone thoracolumbar sympathectomy were studied to determine the source of the urinary catecholamine excretion. Urine extracts were obtained by adsorption of the catecholamines on precipitated aluminum hydroxide, followed by elution, desalting and concentration in vacuo. The urine extracts were assayed by the cat blood pressure method and the values expressed as nor-epinephrine equivalents.

The urinary excretion in twenty-four hours ranged from 14 to 41 μg. nor-epinephrine equivalents in the normotensive group (thirteen subjects) and 7 to 100 μg. nor-epinephrine equivalents in the group of essential hypertensive vascular disease (thirty-five subjects). The distribution is apparent from Table I.

TABLE II

URINARY EXCRETION OF CATECHOLAMINES IN SIXTEEN CASES OF PHEOCHROMOCYTOMA

NO.	NAME	AGE	SEX	HYPERTENSION		URINARY EXCRETION					
				PERSISTENT	PAROXYSMAL	URINE VOLUME	PHOTOFLUORESCENCE URINE EXTRACT 1:100 0.02 ml 0.50 PC-2			PAPER-CHROMATOGRAPHY	BIO-ASSAY CAT'S B.P NOREPI EQUIVALENT
						24 hr. ml.	B_1	B_2	B_3		24 hr. μg
1	A K	48	M	210/115		2850			820	+	1020 Epi 2040 μg
2	E M	42	F	230/140		680			752	+	1700
3	E S	28	M	200/130		1080			640	+	2700
4	A O	39	M	210/140		1810	156	211	432	+	760
5	P P	33	M	190/120–158/124		1200	120	140	284	+	600
6	P F	17	F	180/140	+	1230	530	620	1248	+	1845
7	A M	55	F	140/90–210/110	+	920			860	+	1380 μg Norepi + 920 μg Epi
8	M Gr	49	F	250/150–140/86		1575	180	96	270	+	870
9	Ho Ni	49	M		142/92 ↓300/170	4250	82	76	162	+	1530
10	T M K	45	M		130/84 ↓270/170	2490	365	440	920	+	1120
11	G M C	65	M		130/80 ↓280 160	1080			216	–	290
12	R H	31	F		138/88 ↓220/110	545	137	123	236	+	55* Epi 110 μg
13	A F	26	M		120/80 ↓260/180	900	626	890	1694	+	900 μg Norepi + 900 μg Epi
14	A Z	33	F		126/80	1680	128	172	420	+	590
15	W Gr	42	M		120/80 ↓190/100	940	111	144	280	+	190
16	L F	34	F	225/130–188/118		1350	72	52	138	+	675

* No spontaneous paroxysms observed.

The sixteen cases of pheochromocytoma studied showed a varying clinical picture. They can be subdivided into two main groups: (1) nine cases of persistent hypertension (Cases 1 to 8 and 16) and (2) seven cases of paroxysmal hypertension (with normal blood pressure between attacks) with and without paroxysms during the period of urine collection (Cases 9 to 15).

Two of the cases of this group showed a degree of blood pressure fluctuation beyond the range ordinarily observed in cases of essential hypertensive vascular disease (Cases 7 and 8). Cases 6 and 7 showed superimposed paroxysms not observed on the day of the urine collection.

TABLE III

PHARMACOLOGIC TESTS IN SIXTEEN CASES OF PHEOCHROMOCYTOMA

NO.	NAME	FBS mg%	BMR %	SERUM CHOLEST.	PHARMACOL. TESTS			
					BENZODIOXAN	REGITINE	HISTAMIN	ETAMON
1	A.K.	260	+29 + 1	355	↑230/130 ↓155/ 75			
2	E.M.	235	+61 +40	445	↑220/140 ↓130/ 88	↑(2mg. i.v.) ↓130/ 78		
3	E.S.	norm	+29		↑170/140 ↓130/ 96	↑160/120 ↓136/100		
4	A.O.	94	+33	192	↑190/135 ↓150/100	↑195/130 ↓150/100		
5	P.P.	119 127 136	+22	185	↑206/120 ↓174/104	↑210/120 ↓170/ 92		
6	P.F.	98 (glut)			160/130–180/140 ↓130/ 80			
7	A.M.	209	+26				↑140/ 90 ↓300/180	↑160/100 ↓300/180
8	M.Gr.	norm.	+90hpl. +52lbp.		↑230/118 ↓144/ 60	↑(5mg i.v.) 250/130 ↓146/ 70	↑174/108 ↓260/150	
9	Ha.Ni.	par. 296	− 9 + 1	375	↑300/150 ↓180/ 75			
10	T.M.K.	par. 145		350	↑270/180 ↓180/ 90		↑138/ 84 ↓300/200	
11	G.M.C.						↑120/ 80 ↓280/160	
12	R.H.	par. 176					↑100/ 60 ↓210/150	
13	A.F.	128		260			↑140/ 80 ↓300 210	
14	A.Z.						↑122/ 76 ↓210/130	
15	W.Gr.	130					+	+
16	L.F.	101 143	+18	262	negative	negative	negative	

In the first group the urinary catecholamine excretion is far above that of the normotensive and essential hypertensive group. It ranges from 600 to 2,700 μg. nor-epinephrine equivalents in twenty-four hours. (Table II.) Since the excretion of catecholamines in the essential hypertensive group ranged from 7 to 100 μg., with an average of 27 μg. in thirty-five cases, the differentiation appears clear-cut. If one uses 100 μg. as the upper limit (only once encountered in our group of thirty-five cases of essential hypertension), the catecholamine excretion in our first pheochromocytoma group (persistent hypertension) is 6 to 27 times higher.

Cases 4, 5 and 16 most closely simulated the clinical picture of essential hypertensive vascular disease. A clinical differentiation was impossible in Case 16, who also showed false negative responses to benzodioxan, regitine® and histamine. (Table III.) The urinary catecholamine excretion of this patient was 675 μg. nor-epinephrine in twenty-four hours. The other cases of this group (Cases 1, 2, 6 and 7) presented the pattern of hypertension wi h hypermetabolism and hyperglycemia. Hypermetabolism was also present in Cases 3 and 8 but not accompanied by hyperglycemia. (Table III.)

The excretion calculated per single ml. of extract (=100 ml. urine) did not exceed 6 μg. in the essential hypertensive group and ranged from 42 to 250 μg. nor-epinephrine equivalents

in the first pheochromocytoma group (Case 1 excreted 72 μg. epinephrine/ml.). (Table IV.) These observations justify such chemical screening methods using a constant urine volume, without introducing a volume factor.

In the group of paroxysmal hypertension due to pheochromocytoma, in seven cases (Cases 9 to 15) the urinary excretion of catecholamines ranged from 190 to 1,530 μg. nor-epinephrine equivalent. (Table II.) Case 12 excreted 110 μg. epinephrine which is far in excess of the epinephrine excretion found in normotensives and in cases of essential hypertension. In none of these cases did severe or long-lasting paroxysms occur at the time of urine collection. It is noteworthy that in Case 9 only one mild and short paroxysm was observed at the time of urine collection. This twenty-four-hour specimen contained 1,530 μg. nor-epinephrine equivalent. There was no objective or subjective evidence of a paroxysm during the urine collection in Cases 11, 14 and 15.

TABLE IV

URINARY EXCRETION OF CATECHOL-AMINES IN SIXTEEN CASES OF PHEOCHROMOCYTOMA, DETERMINED BY BIO-ASSAY ON THE CAT'S B.P.

No.	Name	Norepinephrine equivalent/ml
1	A .K.	36 μg = 72 μg Epi
2	E. M.	250 μg
3	E. S.	250 μg
4	A. O.	42 μg
5	P. P.	50 μg
6	P. F.	150 μg
7	A. M.	150 μg Norepi + 100 μg Epi
8	M. Gr.	55 μg
9	Ha. Ni	36 μg
10	T.M.K.	45 μg
11	G.M.C.	26 μg
12	R. H.	10 μg = 20 μg Epi
13	A. F.	100 μg Norepi + 100 μg Epi
14	A. Z.	35 μg
15	W. Gr.	20 μg
16	L. F.	50 μg

If the urinary catecholamine excretion in this second group of pheochromocytoma, normotensive between paroxysms, is compared with the normotensive group, the excretion appears 4 to 30 times higher; if compared with the essential hypertensive group, 2 to 15 times higher.

One might suspect the lowest excretion values to be found in the patients who had no paroxysms during the urine collection, e.g., Case 11, 290 μg. nor-epinephrine; Case 15, 190 μg. nor-epinephrine, but this was not true for Case 14 who excreted 590 μg. nor-epinephrine equivalent without paroxysms. This value is comparable to the twenty-four-hour excretion of a case of persistent hypertension due to pheochromocytoma (Case 5, 600 μg. nor-epinephrine).

In the paroxysmal pheochromocytoma group the urinary excretion calculated per ml. extract (=100 ml. urine) ranged from 20 μg. nor-epinephrine and 20 μg. epinephrine to 100 μg. nor-epinephrine + 100 μg epinephrine. (Table IV.)

Of the sixteen cases of pheochromocytoma studied, fifteen proved positive when studied by bio-assay (cat blood pressure) alone; Case 12 was negative when studied by this method alone. (Table V.)

1b. Paper Chromatography of Urine Extracts. Method:[2,23] The catecholamine content of the urine extracts has been studied with the aid of ascending one-dimensional paper chromatograms. These are developed in a cylindrical glass jar fitted with a glass stand from which are suspended the paper sheets. The developing solvent used is a mixture of 98 cc. water-saturated phenol, 2 cc. 88 per cent phenol, and 0.1 per cent (w/v) 8-hydroxyquinoline. The mixed solvent is poured into the bottom of the glass jar before suspending the papers therein. At the same time a small beaker containing 30 ml. of water and 5 ml. conc. HCl is placed in the jar. The apparatus is covered with close-fitting glass plates in order to permit the enclosed atmosphere to become saturated with the solvent vapors.

Application of the samples: Whatman No. 1 filter paper was used throughout this work. After allowing a margin of at least 1.5 cm. at each side of the sheet, the remaining portion is divided into the number of lanes required; each lane should be 3 cm. wide or more. A pencilled dot in the center of each lane, placed 8 cm. from the lower edge, marks the point at which the samples are applied; 0.05 ml. of an acid-alcohol extract (cf. p. 313) (the extract represents a 100-fold concentration) is applied, in 0.0025 ml. increments, to a given spot. A Gilmont ultra-microburette is used to apply the samples to the paper. Whenever successive drops are applied to the same spot, each is permitted to dry at room

temperature before adding the next. A standard solution of epinephrine and nor-epinephrine is also applied in one of the lanes; this solution is freshly prepared by dissolving the catecholamines in a drop of water, then making to volume in acid-alcohol (99 ml. 95 per cent ethanol, 1 ml. conc. HCl), with 1 ml. of solution containing 1.0 mg. of each free base. The standard solution may also be prepared by dissolving the amines in a urine acid-alcohol extract previously shown not to contain appreciable quantities of epinephrine and nor-epinephrine. It is desirable to have, as a minimum, 2 to 3 μg. of each base in the standard lane.

TABLE V

DIAGNOSTIC STUDIES IN SIXTEEN CASES OF PHEOCHROMOCYTOMA

NO.	NAME	HYPERTENSION		URINARY EXCRETION OF CATECHOL-AMINES			PHARMACOL TESTS	
		Persistent	Paroxysmal	Fluo-rescence	Paper-chromat.	Bio-assay cat's B.P.	Benzodioxan	Histamin
1	A.K.	+		+	+	+	+	
2	E.M.	+		+	+	+	+	
3	E.S.	+		+	+	+	+	
4	A.O.	+		+	+	+	+	
5	P.P.	+		+	+	+	+	
6	P.F.	+	+	+	+	+	+	
7	A.M.	fluct.	+	+	+	+		+
8	M.Gr.	fluct.		+	+	+	+	+
9	Ha.Ni		+	+	+	+	+	
10	T.M.K.		+	+	+	+	+	+
11	G.M.C.		+	+	−	+		+
12	R.H.		+	+	+	−		+
13	A.F.		+	+	+	+		+
14	A.Z.		+	+	+	+		+
15	W.Gr.		+	+	+	+		+
16	L.F.	+		+	+	+	−	−

Development of the chromatograms: When the standard and unknown spots are thoroughly dry (one hour) the paper sheets are suspended in the previously prepared jar so that about 1 cm. of the lower edge is submerged in the solvent. Paper chromatograms are developed in the phenolic solvent until the solvent front has travelled 22 to 27 cm. beyond the sample spots. This usually requires fourteen to sixteen hours.

Identification of epinephrine and nor-epinephrine in the developed chromatograms: The developed chromatograms are dried for one hour at room temperature in a hood with a strong draft after which they are sprayed with 0.44 per cent $K_3Fe(CN)_6$ in 0.2M phosphate buffer, pH 8.3.[24] One μg. quantities of epinephrine and nor-epinephrine can be identified by their relative positions and the distinctive peach, pink-lavender tints of their respective reaction products. On a paper chromatogram developed in phenol as described, epinephrine has the higher R_f of the two catechols. Pink spots which appear in the urine extract are assumed to represent epinephrine or nor-epinephrine when their tints and relative positions are similar to those of the standards.

A pink-lavender spot often is indicated in most developed urine extracts at a point midway between epinephrine and nor-epinephrine, corresponding to the R_f and color of hydroxytyramine standards.

1b. Paper Chromatography. Results: By applying 0.05 ml. of an acid-alcohol urine extract to paper, as described in *1* and *1b*, pheochromocytoma with persistent hypertension or of the paroxysmal type can be easily distinguished from essential hypertensive vascular disease. The smallest amount of nor-epinephrine or epinephrine easily demonstrable on paper by the use of the described indicator is 1 μg. The highest nor-epinephrine content found in urine extracts of essential hypertensives was 6 μg. per ml. which makes the amount contained in 0.05 ml. (0.3 μg.) invisible on paper. Urine extracts from patients with pheochromocytoma and persistent hypertension in this series, on

the other hand, contained 42 to 250 μg. nor-epinephrine or 72 μg. epinephrine per ml. The urine extracts of patients with paroxysmal hypertension due to pheochromocytoma contained from 20 μg. nor-epinephrine or epinephrine to 100 μg. epinephrine and 100 μg. nor-epinephrine per ml. Of sixteen patients with pheochromocytoma studied by us fifteen gave positive findings by paper chromatography. In one case (Case 11) the extracts gave unsatisfactory chromatograms; this despite the fact that the nor-epinephrine content per ml. was above the threshold of the method. (Tables III, IV and V.)

1c. Photofluorometric Evaluation. Method: Lund has studied the quantitation of adrenaline by photofluorometry.[25] In our studies of total fluorescence of urine extracts we have used essentially his adrenaline procedure for developing the fluorescence, as follows: Oxidation with MnO_2 at pH 6 is followed by treatment with a sodium hydroxide-ascorbic acid solution. This fluorescence is compared with that obtained without ascorbic acid. In the latter case the fluorescence of epinephrine disappears within a few minutes while that of nor-epinephrine disappears in twenty-five minutes. When the ascorbic acid is added to the sodium hydroxide, the fluorescence is stable. It is this property which permits differentiation of epinephrine and nor-epinephrine from most other substances in the extracts which exhibit fluorescence in alkaline media.*

Our procedure is as follows: 0.02 ml. and 0.005 ml. aliquots of the extract are delivered in duplicate into 15 ml. glass-stoppered centrifuge tubes. The volume of fluid in each tube is made up to 7 ml. with distilled water, then 1 ml. of a 1 M pH 6 acetate buffer is added. About 50 mg. of manganese dioxide† is added from the tip of a spatula. The tubes are stoppered and inverted repeatedly for sixty seconds. The tubes are then centrifuged at 1,500 r.p.m. for one minute; the supernatants are decanted into the photoflurometer cuvettes. To one cuvette of each pair 1 ml. of 5 N sodium hydroxide is added and the contents are immediately mixed. These cuvettes are allowed to stand twenty-five minutes before reading. To the other cuvette of each pair is added 1 ml. of a solution prepared by adding 5 ml. of 5 N sodium hydroxide to 1 ml. of 1 per cent aqueous ascorbic acid solution. The tubes are thoroughly shaken before the fluorescence is measured. These tubes are read in four minutes, at which time maximum development of fluorescence usually has been reached. The sodium hydroxide-ascorbic acid solution should not be allowed to stand longer than ten to fifteen minutes before use. The corrected fluorescence value of a given specimen (obtained by subtracting that measured with sodium hydroxide from that measured with sodium hydroxide-ascorbic acid) is small if little or no epinephrine or nor-epinephrine is present.

A Coleman model 12B photofluorometer modified to include a 931-A photomultiplier tube has been used for these determinations, employing a B-3 primary lamp filter and a PC-2 secondary photocell filter for the catecholamine fluorescence measurements. The sensitivity of the meter is set to read 50 on a scale of 100 for quinine at a concentration of 0.10 μg. per ml. in 0.1 N sulfuric acid, employing B-1, PC-2 filters.*

The determination requires readings with the B-1 and B-2 primary filters as well as with the B-3 filter. The purpose of this is to screen out occasional urine specimens in which the non-specific fluorescence is so high as to make the determination unreliable. For pure epinephrine the ratio of the B-1 reading to the B-2 reading is 0.7. For pure nor-epinephrine it is 2.0. With urine specimens the value has ranged from 0.7 to 8.0. We suggest that specimens in which a high B-3 fluorescence is accompanied by a B-1/B-2 ratio in excess of 2 do not owe their fluorescence to the presence of significant quantities of epinephrine or nor-epinephrine.

1c. Photofluorometric Evaluation. Results. In our experience the differentiation of pheochromocytoma from essential hypertensive vascular

* We have not been able to obtain satisfactory results with Lund's method for quantitation of mixtures of nor-epinephrine and epinephrine by differential oxidation at pH 3 and pH 6.5.[26]

† Seven commercial samples of this reagent were tested. The MnO_2 which was found suitable from the point of view of suspending well on shaking and packing well on centrifuging was that sold by Coleman and Bell Co., Norwood, Ohio (Reagent Grade).

* The PC filter is placed between the photocell and the sample. The filter used by us, PC-2 Coleman (Corning No. 3486), filters out wave lengths below 530 mμ.

The function of the B filter is to pass light of the wave-length required to excite the sample. B-1 (Coleman) (Corning No. 5874) passes the 365 mμ line. B-2 (Coleman) is a combination filter, Corning No. 5113, half standard thickness and Corning No. 3389 adjacent to the light; this passes the 436 mμ line. B-3 (Coleman) (Corning No. 5113) passes part of the 436 mμ line and all of the 405 mμ.

disease can best be achieved by photofluorometric evaluation of the urine extracts obtained by adsorption on precipitated aluminum hydroxide (Method *1*). This method does not permit separate quantitation of epinephrine and nor-epinephrine in the urine extracts but is limited to total fluorescence values at pH 6.0. The highest specific readings for epinephrine and nor-epinephrine are obtained with the B-3 filter. B-1 and B-2 readings are necessary to screen out non-specific fluorescence in the urine extracts. In pheochromocytoma B-3 readings are always higher than those of B-1 or B-2, and the ratio B-1:B-2 lies between 0.7 and 2.0, which represents the ratios for epinephrine and nor-epinephrine, respectively. (Table II.)

The B-3 readings for 0.02 ml. urine extract (100:1) ranged from 138 to 1,694 in our sixteen cases of pheochromocytoma (groups I and II). These readings do not parallel the nor-epinephrine equivalent per ml. obtained by bio-assay (Table IV), since the fluorescence of epinephrine is four times greater than that of nor-epinephrine. The variable epinephrine/nor-epinephrine ratio of the urine extracts parallels that of the tumors. (Table VI.)

The B-3 readings for 0.02 ml. extracts of forty-seven cases of essential hypertensive vascu-

TABLE VI

CHEMICAL TUMOR ANALYSES IN SIXTEEN CASES OF PHEOCHROMOCYTOMA

NO.	NAME	TUMOR				
		WEIGHT	EPI-NEPHRINE	NOREPI-NEPHRINE	EPI-NEPHRINE	SITE
		g	mg/g	mg/g	%	
1	A.K.	43	5.9	1.85	76	adrenal
2	E.M.	140	0.5	4.1	11	"
3	E.S.	93.5	trace	5.22	TRACE	"
4	A.O.	lt. 75 rt. 82	3.59 2.89	6.02 3.18	37 47	" "
5	P.P.	50	0.96	3.12	23	"
6	P.F.	40	4.9	7.7	39	"
7	A.M.	274	7.2	9.0	44	"
8	M.Gr.	43	0.42	5.1	8	"
9	Ha.Ni	17	1.7	7.6	18	"
10	T.M.K.	56	3.2	7.3	30	"
11	G.M.C.	45	8.7	1.9	82	"
12	R.H	56 (+blood 130)	7.22	1.84	80	"
13	A.F.	225.5	8.42	5.48	60	extra adrenal
14	A.Z.	30	1.47	3.67	29	adrenal
15	W.Gr	57	3.87	3.56	52	"
16	L.F.	7.7	0.35	1.82	16	"

lar disease ranged from 2 to 44. The distribution is apparent from Table VII.

The full set of readings of the five cases of essential hypertension showing the highest B-3 readings, 34–44, are given in Table VIII.

TABLE VII
DISTRIBUTION OF B-3 READINGS IN FORTY-SEVEN CASES OF ESSENTIAL HYPERTENSIVE VASCULAR DISEASE

B-3 PC-2 readings (Q = 50)	2–10	11–20	21–30	31–40	41–44
No. of cases.....	7	22	13	3	2

TABLE VIII
FLUOROMETRIC READINGS IN FIVE CASES OF ESSENTIAL HYPERTENSIVE VASCULAR DISEASE

Case	B-1	B-2	B-3	B-1/B-2
A. M.	80	10	44	8.0
D. K.	29	19	41	1.5
E. D.	73	12	40	6.0
R. R.	68	10	34	6.8
N. S.	36	15	34	2.4

In four of these cases B-1 is greater than B-3, and the B-1/B-2 ratio is greater than 2.0, ranging from 2.4 to 8.0. This excludes epinephrine or nor-epinephrine as the predominant source of fluorescence in these urine extracts. D. K. is the only case in this group whose urine extract may derive its fluorescence from nor-epinephrine predominantly. However, the lowest B-3 reading in the pheochromocytoma series of sixteen cases is more than three times higher than that of this case.

1d. Absolute Quantitation of Epinephrine and Nor-epinephrine: Method. A urine extract prepared in 5 per cent acetic acid and representing a 500-fold concentration is prepared and this extract is applied to paper and a chromatogram developed according to the method of James.[24] Eluates of the separated epinephrine and nor-epinephrine are quantitated fluorometrically. The details of our procedure are as follows:

Preparation of the extract: A 525 cc. portion of a twenty-four-hour urine sample is hydrolyzed and worked up to the acetone-alcohol stage exactly as previously described. The acetone-alcohol solution in the 25 cc. pear-shaped flask is concentrated to less than 0.5 ml., and the residue is taken up in 5 per cent acetic acid and made to volume in a 1 cc. Krumholz microvolumetric flask. The solution is then transferred to a small test tube and centrifuged, giving a clear brown solution. The clear supernatant is used as soon as possible for chemical quantitation.

Develcpment of the paper chromatogram and preparation of the eluates: A descending, one dimensional paper chromatogram is run, using Whatman No. 1 filter paper sheets, 10 cm. by 46 cm. The sheets are ruled into lanes 3½, 3 and 3½ cm. wide; solutions are applied at the center of the middle lane and 1½ cm. on the outside of each of the ruled lines; the pencilled dots on which the solutions are placed are marked 9 cm. from the top of the paper. Using a glass 1-lambda micropipette, a total of 0.0050 ml. of a given extract is applied to each spot. To the outside spots, after drying (fifteen minutes), is applied as little as 0.7 gamma each of epinephrine and nor-epinephrine in 5 per cent acetic acid solution.

The spots are allowed to dry for one hour, the top of the paper then being folded into the glass trough which has been placed on top of the steel rack in the cylindrical jar.* The developing solvent is prepared as follows: 40 parts of n-butanol, 10 parts glacial acetic acid and 50 parts water are thoroughly mixed in a separatory funnel; the bottom layer is drawn off and the top layer is placed in a 250 ml. centrifuge bottle and refrigerated for one hour. The resultant mixture is centrifuged at 1,800 r.p.m. and the top layer is decanted into a beaker. This is carefully poured into the glass trough into which the top of the paper has already been folded. The chromatogram is allowed to develop for twenty-two hours in a temperature-controlled room set at 22°C. The papers are carefully removed from the jar, hung in a hood and dried for one hour in a strong current of air. The center lane is then cut out and the outside lanes are sprayed with 0.44 per cent $K_3Fe(CN)_6$ in 0.2M phosphate buffer, pH 8.3. The indicated epinephrine and nor-epinephrine spots are outlined with pencil and those portions of the center lane containing the corresponding areas are cut out, accordion-folded, and placed in 4 inch test tubes. To each test tube is added 8 cc. 0.01 N HCl which is sufficient to cover the enclosed paper strip. The

* A cylindrical glass jar containing a stainless steel rack which supports the solvent trough and the paper has been found very useful for the development of the chromatogram (University Apparatus Co., Berkeley, California).

strips are allowed to soak from one hour to overnight.

Fluorometric quantitation of the eluates: The liquid in each tube is carefully pipetted off and transferred to a 50 cc. beaker containing a small magnetic stirring bar. The paper and test tube are carefully washed with two or three 5 cc. portions of water which are combined with the original eluate. The combined eluate and washes are adjusted* to pH 3.–3.5 with 0.5 N NaOH and to pH 6.–6.5 with 0.01 N NaOH using a line operated pH meter and a magnetic stirrer. The solution is then made to volume with water, in a 50 ml. volumetric flask for nor-epinephrine, and a 100 ml. flask for epinephrine. Twenty ml. of the solution is placed in a 40 ml. glass-stoppered centrifuge tube and 2 cc. of pH 6.5 1 M acetate buffer† is added. Approximately 0.2 gm. MnO_2 is added and the tube is inverted for twenty seconds. The resulting mixture is centrifuged for one minute and the supernatant filtered by suction through a Hirsch funnel. Eight ml. portions of the filtrate are added to each of two photofluorometer cuvettes, and the fluorescence of these solutions is developed and measured in the same manner as in the screening method (*1c*). To cuvette No. 1 is added 1 cc. of a fresh NaOH-ascorbic acid solution; to cuvette No. 2 is added 1 cc. of 20 per cent NaOH (the latter tube represents the blank). Both tubes are thoroughly shaken and the reading of tube No. 1 is taken in one minute (epinephrine) or four minutes (nor-epinephrine); readings are taken with the B3-PC2 filters on the fluorometer standardized to read 50 (on a scale of 100) for quinine at a concentration of 0.1 μg./ml. 0.1 N H_2SO_4 employing B-1, PC-2 filters. From the readings obtained are subtracted those given (in twenty-five minutes for nor-epinephrine and five minutes for epinephrine) by the cuvettes containing no ascorbic acid (blanks). The corrected readings are then compared to standard straight line graphs for epinephrine and nor-epinephrine.

Standard graphs for epinephrine and nor-epinephrine: Standard aqueous solutions of epinephrine, containing 0.04 to 0.20 μg. per 10 cc., and standard solutions of nor-epinephrine varying in concentration from 0.1 to 0.5 μg. per 10 cc. of water, are freshly prepared. Each of two 10 cc. portions for a given concentration is treated with 1 cc. pH 6.5 1 M acetate buffer, and 0.1 gm. MnO_2. The fluorescence of 8 cc. of each supernatant, obtained following inversion, centrifuging and filtering is determined in the manner already described. (Figs. 2 and 3.)

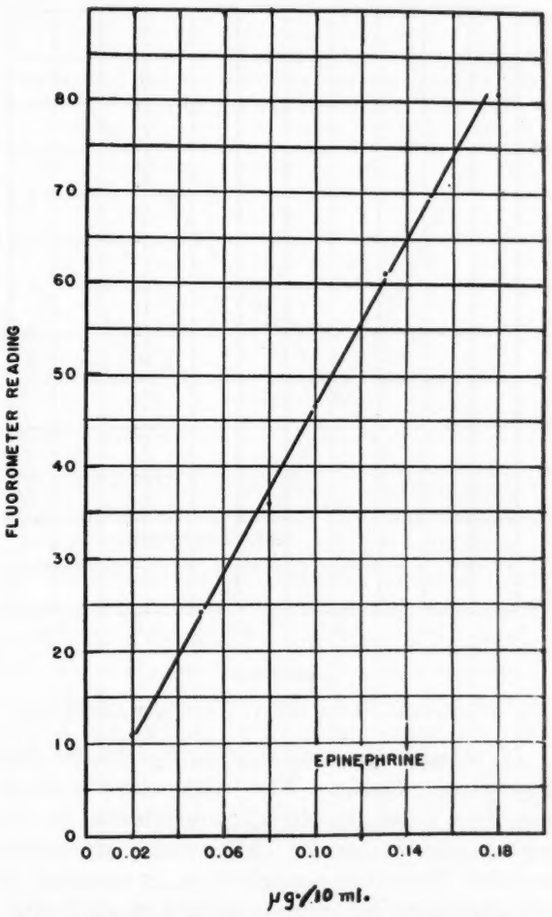

FIG. 2. Standard fluorescence curve for epinephrine.

Over-all recovery of epinephrine and nor-epinephrine in chemical assay procedure: To test the efficiency of the entire analytical procedure in urine samples, known amounts of epinephrine and nor-epinephrine were added to normal urine shown by bio-assay or chemical assay to contain 1 per cent or less of the added amounts. The urine was hydrolyzed, treated with $Al_2(SO_4)_3$ and an extract prepared and quantitated as previously outlined. Recovery results are as follows:

Experiment 1: To 500 cc. of urine containing 3.0 μg. nor-epinephrine equivalent as determined by bio-assay was added a mixture of 400 μg. epinephrine and 1,400 μg. nor-epinephrine. The recovery of epinephrine was 85, 87 per cent, of nor-epinephrine 97, 108 per cent.

* The pH may be adjusted directly by adding, with shaking, 5 ml. of 1M Na acetate to the volumetric flask.

† 2 ml. of H_2O is substituted if pH adjustment has been carried out with 5 ml. 1 M Na Acetate as in footnote. *

Experiment II: To 500 cc. of urine containing 2.0 μg. epinephrine and 10.0 μg. nor-epinephrine, determined by chemical assay, was added a mixture of 300 μg. epinephrine and 1,000 μg. nor-epinephrine. The recovery for epinephrine was 95 per cent, for nor-epinephrine 95 per cent.*

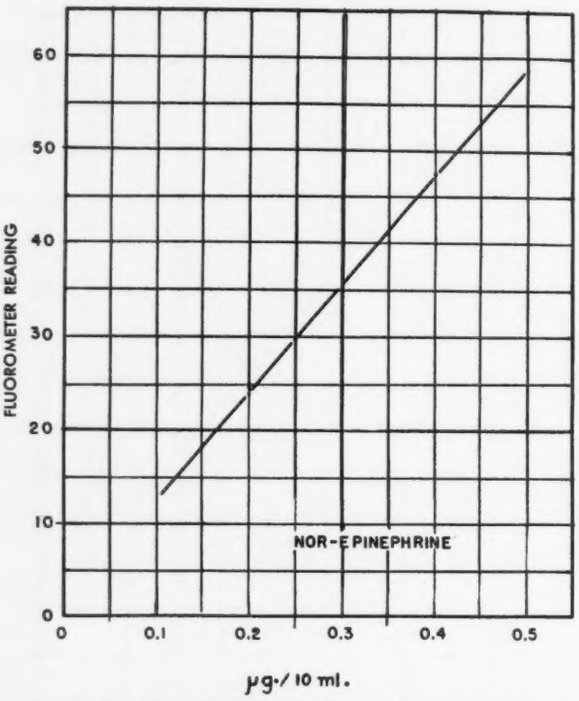

FIG. 3. Standard fluorescence curve for nor-epinephrine.

1d. Absolute Quantitation of Epinephrine and Nor-Epinephrine: Results. The urine extracts of the last five cases of pheochromocytoma in this series were studied by this recently developed method. Recovery experiments, as recorded in the preceding paragraph, proved so satisfactory that an evaluation of the bio-assay procedure seemed feasible. (Table IX.)

The nor-epinephrine values obtained by chemical assay were higher in Cases 4, 5, 14 and 15 than the nor-epinephrine equivalents obtained by bio-assay by the cat blood pressure method. The 8 per cent difference observed in Case 16 is within the limits of error of the bio-assay method. The difference between the results of the two methods in the first four cases is even more striking if one attempts to calculate the expected bio-assay figure by adding one-quarter to one-half of the chemical epinephrine to the nor-epinephrine figure—this because the pressor action of 2 to 4 parts of epinephrine was equivalent to one part nor-epinephrine in these experiments. In Cases 4, 5 and 14 the bio-assay figure actually obtained is 31 to 37 per cent smaller, in Case 15 more than 50 per cent smaller. A biphasic response of cat blood pressure to small epinephrine quantities cannot account for the difference, since it was absent in part of the experiments. Racemization during acid hydrolysis seems the most likely explanation for the loss of biological activity of nor-epinephrine and epinephrine in the urine extracts. This phenomenon was reproduced in the following experiment:

* Below 50 μg. nor-epinephrine, the recovery is irregular.

TABLE IX
TWENTY-FOUR-HOUR URINARY EXCRETION OF CATECHOLAMINES IN FIVE CASES OF PHEOCHROMOCYTOMA

Case	Method of Assay	
	Chemical (μg.)	Biological (Nor-epinephrine equiv.) (μg.)
A. O., II	Nor-epinephrine 890 Epinephrine 456	700
P. P.	Nor-epinephrine 820 Epinephrine 290	600
A. Z., I	Nor-epinephrine 724 Epinephrine 405	570
W. Gr., I	Nor-epinephrine 425 Epinephrine 192	193
L. F.	Nor-epinephrine 616 Epinephrine 41	675

One hundred μg. nor-epinephrine were added to 50 cc. urine (pH 2.5) shown to contain less than 1 μg. nor-epinephrine. Determination by bio-assay showed 1 cc. to be equivalent to 1.9 μg. ±0.1 μg. nor-epinephrine. If the urine specimen with added nor-epinephrine was kept at pH 2.5 and hydrolyzed by heating on a steam bath for thirty minutes at pH 1.5, 1 cc. became equivalent to 1.5 μg., showing a loss of 22 per cent. If the urine specimen was kept and hydrolyzed at pH ≦ 1, 1 cc. contained 1.0 μg., showing a loss of 43 per cent. In a second experiment the loss amounted to 50 per cent. All samples were adjusted to pH 4-6 immediately before the bio-assay.

2. Rapid Screening Procedure for Pheochromocytoma: Method

Lund's procedure[25] for gross isolation of catecholamines from plasma was found to be

useful as a rapid preliminary step in the fluorometric screening of urines. The differentiation between pheochromocytomas and essential hypertensives, however, is not as clear-cut with this procedure as was found true in the longer method; in cases giving a positive result procedure *1c* is used as a check.

Lund stated that he has used a similar procedure for the quantitation of epinephrine and nor-epinephrine in plasmas and urines of pheochromocytoma patients.[27] We have not been able to confirm these quantitation studies, one of the reasons being that the total fluorescence obtained is due only partly to the presence of epinephrine and nor-epinephrine. We are using the following procedure to determine the total fluorescence of acetic acid eluates from alumina columns previously treated with hydrolyzed urine:

In a glass tube (0.8 cm. internal diameter and drawn to a narrow opening at the lower end) is placed a wad of glass wool. One gm. of alumina* in aqueous suspension is poured in, and a small glass wool pad is placed on the column. The tube is connected to suction using a suction flask and the column is washed with 30 cc. of H_2O followed by 5 cc. of 0.2 N sodium acetate. The flow of liquid off the column should be approximately 1 to 2 drops per second. The tube is now stoppered until the urine sample is ready; to prevent the column from drying a small layer of liquid always should be allowed to remain above the solid.

Fifty cc. of urine in a 200 cc. Erlenmeyer flask is adjusted to pH 1 to 1.5 with hydrion paper and heated on a steam bath for one-half hour. The urine is cooled, then approximately 20 cc. is poured into a 30 cc. beaker containing a small magnetic stirring bar. The urine is titrated with 5 N NaOH using the line-operated pH meter to pH 7.5 to 7.6. The solution is centrifuged (a flocculent precipitate sometimes forms) in a 40 cc. centrifuge tube for about one minute. Ten cc. of supernatant is allowed to pass through the column followed by small portions of 0.2 N sodium acetate (total of 5 cc.). While the column is still under some sodium acetate solution the glass tube is removed from the suction flask and the bottom end of the tube is rinsed with distilled water. The tube is clamped above a 50 cc. beaker containing a small magnetic stirring bar. Ten cc. of 0.2 N acetic acid is added to the tube and allowed to flow by gravity; 10 cc. of water is then allowed to flow through the column. The total aqueous acid eluate is titrated to pH 6.5 with saturated Na_2HPO_4 using a line-operated pH meter and a magnetic stirrer. This solution is made up to 50 cc.

* Activated alumina, mesh 80–200; manufactured by Aluminum Ore Co.

TABLE X

FLUOROMETRIC READINGS IN SEVEN CASES OF PHEOCHROMOCYTOMA USING THE RAPID SCREENING METHOD

Case	B-1	B-2	B-3	B-1/B-2
E. S.	340	210	620	1.4
A. O.	250	260	540	0.9
P. P.	270	230	530	1.2
M. Gr.	190	125	330	1.5
A. Z.	155	150	315	1.0
W. Gr.	225	195	415	1.1
L. F.	180	100	270	1.8

To each of two 15 cc. glass-stoppered centrifuge tubes containing 6 cc. of H_2O is added 2 cc. of eluate. Approximately 0.1 gm. of MnO_2 is added, the tubes are inverted, and the total fluorescence is then determined in the same manner as in method (*1c*). In this rapid procedure, however, the blank to be subtracted from the sodium hydroxide-ascorbic acid-treated solution consists of the readings taken on the same cuvette before the addition of the basic reagent. The corrected readings are then multiplied by 5 to make them comparable to those found by method (*1c*), corresponding to 2 cc. urine or 0.02 ml. extract.

In cases giving a positive result the readings may be off the scale of the fluorometer, so the experiment is repeated using 1 cc. of eluate and 7 cc. of water. The corrected reading is then multiplied by 10.

2. Rapid Screening Procedure: Results. Urine specimens of seven cases of pheochromocytoma were examined using this method. The B-3 readings calculated for 2 ml. urine were found to range from 270 to 620. The complete set of readings for these cases is given in Table x.

There is no doubt that this adsorption procedure is less specific than the long method (*1* and *1c*). In contrast to the long method (*1c*) which gave 44 as the highest B-3 readings in the group of essential hypertension for 0.02 ml. urine extract, with a B-1/B-2 ratio of 8.0, the B-3 readings of the same group ranged as high as

110 using the short method, considering only B-3 readings which were higher than B-1 or B-2 and showed a B-1/B-2 ratio between 0.7 and 2.0. (Table XI.) A single case of essential hypertension in a child showed a reading of:

B-1	B-2	B-3	B-1/B-2
75	50	205	1.5

The same urine specimen using the long method (*1c*) gave readings of:

B-1	B-2	B-3
20	9	20

Insofar as the rapid screening procedure is concerned, Case 1 is considered a false positive. We therefore recommend that any urine sample which gives B-3 readings greater than 110 and has a B-1/B-2 ratio of less than 2 be tested by the long procedure (*1c*). The rough screening test is suitable for mass screening of hypertensives but a positive result has to be checked by the more specific method.

Table XI shows the findings in a group of forty-six cases of hypertension. Case 1 shows B-3 greater than B-1; B-1 greater than B-2, a B-1/B-2 ratio of 1.5, and has to be regarded as false positive since the long procedure gave a low normal value. Case 2 shows a B-3 of 130 but a ratio of 2.3, and was proved to be negative. Case 3 is considered to show the upper limit of B-3 readings (110) with this method, for a negative urine having a B-1/B-2 ratio ≤ 2. Cases 4 to 11 and 13, 15, 16, 18, 20–27, 29, 33, 35, 36, 40 and 41 can be regarded as negative since B-1 is greater than B-3, and the B-1/B-2 ratio ranges from 2.7 to 20.0.

TABLE XI

FLUOROMETRIC READINGS IN FORTY-SIX CASES OF ESSENTIAL HYPERTENSIVE VASCULAR DISEASE USING THE RAPID SCREENING METHOD

Case No.	B-1	B-2	B-3	B-1/B-2
1*	75	50	205	1.5*
1a	35	15	105	2.3
2	115	50	130	2.3
3	80	45	110	1.7
4	110	40	100	2.7
5	205	20	110	10.0
5a	100	10	70	10.0
6	185	15	110	12.3
7	220	35	140	6.3
8	310	15	130	20.0
9	325	20	155	16.0
10	210	20	105	10.5
11	155	40	115	3.8
12	60	38	78	1.6
13	75	15	50	5.0
14	28	10	33	2.7
15	65	15	55	4.3
16	80	20	75	4.0
17	70	35	75	2.1
18	50	10	40	5.0
19	70	20	70	3.3
20	150	15	80	10.0
21	40	0	0	.....
22	25	0	0	.....
23	75	5	40	15.0
24	120	20	80	6.0
25	55	20	40	2.7
26	100	15	60	6.6
27	75	10	55	7.5
28	10	0	10	.....
29	30	0	5	.....
30	15	25	35	0.6
31	0	0	5	0
32	20	5	20	4.0
33	75	20	60	3.7
34	15	15	25	1.0
35	80	20	30	4.0
36	80	30	75	2.7
37	30	20	30	1.5
38	0	0	5	.....
39	0	5	0	.....
40	80	15	50	5.0
41	115	15	60	8.0
42	5	5	20	1.0
43	15	0	0	.....
44	20	10	30	2.0
45	40	30	60	1.3
46	50	20	50	2.5

* Essential hypertension in a child who showed, on one occasion, the only false positive result in this series.

3. *Pharmacologic Tests*

An evaluation of the results of pharmacologic testing in our group of sixteen cases of pheochromocytoma shows that false negative responses were rarely encountered. Of ten tumor cases tested with benzodioxan[9,10] one showed a false negative response. Of six cases tested with regitine®[11] there was one false negative; of eight cases tested with histamine[28] one was false negative. In a larger group of cases previously analyzed we found an incidence of 5 per cent false negative benzodioxan responses in sixty-two verified cases of pheochromocytoma.[10] The present incidence of one out of ten is closer to our old figure than to reports found in the literature.[29] The higher incidence of false negative responses to benzodioxan in the experience of the Mayo group[29] may be due to faulty technic, e.g., injection time of four minutes instead of two minutes, which weakens the adrenergic blocking

action of benzodioxan beyond the desired limits, and the use of benzodioxan while the patient's diastolic pressure did not exceed 100 mm. Hg.

It is noteworthy that the only false negative response to benzodioxan in this group was found in a patient who also exhibited a false negative response to regitine. (Case 16 in Tables II and III.) This patient also showed hypertension persisting after removal of the tumor, as previously described by us in cases of pheochromocytoma with false negative response to adrenergic blocking agents.[4] The probable mechanism: a "non-humoral" phase of hypertension in pheochromocytoma.

The incidence of false positive pharmacologic responses in ninety-one cases of essential hypertensive vascular disease in our experience follows:

Benzodioxan test: Repeated false positive responses to benzodioxan and regitine were obtained in one patient who showed negative urinary excretion and proved negative on exploration. A second patient showed a false positive response to benzodioxan only when tested for the first time. The benzodioxan test was twice negative on repeated trials and so was the urinary catecholamine excretion.

The incidence of false positive regitine responses in this group is much higher: eight cases of essential hypertension, including the above mentioned case and a patient with uremia not included in this figure. These findings indicate the need for a benzodioxan test or urinary excretion studies in all cases giving a positive response to regitine.

A false positive histamine response was observed twice in our essential hypertension group.

COMMENTS

In patients with pheochromocytoma the urinary excretion of nor-epinephrine and epinephrine has been found consistently to be far in excess of the amounts excreted by normotensive and essential hypertensive subjects. The quantities of catecholamines recovered from these urines represent, however, only a small fraction of the total amount secreted by the tumor. This can be best demonstrated by the model experiment of a nor-epinephrine infusion.[5] Table XII shows the urinary excretion of nor-epinephrine when infused for two to six hours. The percentage excreted in the urine of seven subjects varied from 0.3 to 4.0 per cent. The collection periods prior to the experiment, as well as during and after the infusion, extended for twenty-four hours. The percentage increased with increasing speed of infusion. The amounts infused, e.g., 6,960 μg. nor-epinephrine in four hours, and 7,550 μg. in six hours, are comparable to the amounts secreted by a pheochromocytoma

TABLE XII
URINARY EXCRETION OF NOR-EPINEPHRINE BEFORE, DURING AND FOLLOWING NOR-EPINEPHRINE INFUSION (TWENTY-FOUR-HOUR PERIOD)

Subject	24-Hour Period Prior to Infusion (μg.)	Nor-epinephrine Infused (μg.)	24-Hour Period during and after Infusion (μg.)	Nor-epinephrine Excreted (%)
1	16	1940	21	0.3
2	20	1950	52	1.6
3	15	1950	66	2.6
4	36	1940	89	2.7
5	15	1955	59	3.0
6	12	6960	280	4.0
7	45	7550	255	3.4

(judging from the pressor response). Similar data were independently obtained by von Euler and Luft.[30] The nor-epinephrine recovered from the urine of normal and hypertensive subjects correspondingly represents an as yet unknown but certainly small fraction of the amount secreted by the sympathetic nervous system and the adrenal medulla. This fraction has apparently escaped metabolic breakdown and is excreted partly as sulfate ester and partly in the free form.[12]

The fact that the catecholamine mixture of human urine if tested by bio-assay consists predominantly of nor-epinephrine and contains less than 15 per cent epinephrine[31] suggests that its source is the sympathetic nervous system rather than the adrenal medulla. This assumption is easily proved, since in Addison's disease the urinary excretion, measured by bio-assay as nor-epinephrine equivalent, may be within normal limits.[5,16] The urinary excretion values of ten cases of Addison's disease are listed in Table XIII. The lowest urinary excretion values, on the other hand, were observed in fifteen cases of essential hypertension following thoracolumbar sympathectomy. (Table XIII.)

The evidence obtained in this series of sixteen cases of pheochromocytoma and ninety-one cases of essential hypertensive vascular disease sug-

gests that the photofluorometric evaluation of urinary extracts for catecholamines (Method *1c*) constitutes the most reliable diagnostic test for pheochromocytoma. This is true for cases with persistent hypertension as well as for the paroxysmal type. The most surprising finding was

TABLE XIII
URINARY EXCRETION OF CATECHOLAMINES IN TEN CASES OF ADDISON'S DISEASE AND IN FIFTEEN CASES OF ESSENTIAL HYPERTENSION FOLLOWING THORACOLUMBAR SYMPATHECTOMY

Addison's Disease		Essential Hypertension after Sympathectomy	
Case	24-Hour Excretion Nor-epinephrine Equivalent (μg.)	Case	24-Hour Excretion Nor-epinephrine Equivalent (μg.)
		1	11
1	20	2	4
2	14	3	5
3	84	4	11
4	16	5	14
5	4	6	2
6	43	7	12
7	18	8	10
8	5	9	5
9	14	10	5
10	15	11	2
		12	7
		13	11
		14	15
		15	9

the amount of "leakage" of catecholamines encountered in paroxysmal cases between attacks, i.e., in normotensive periods. To account for the lack of a pressor response to the sizeable amounts of nor-epinephrine secreted by some of the tumors between attacks one feels tempted to speculate that an adrenergic blocking agent may be secreted by the body.

No false negative or false positive tests were observed using the method of adsorption on precipitated aluminum hydroxide plus photofluorometric evaluation. The method using urine adsorption on aluminum oxide columns (Method *2*) permits rapid screening but is less reliable than Method *1c*. When a positive result is obtained, confirmation by the longer and more specific method, *1c*, is required.

SUMMARY

1. The urinary excretion of catecholamines (nor-epinephrine and epinephrine) was studied in sixteen cases of pheochromocytoma, ninety-one cases of essential hypertensive vascular disease, thirteen normotensive healthy subjects, ten cases of Addison's disease and fourteen cases of essential hypertension following thoracolumbar sympathectomy.

2. The source of nor-epinephrine in human urine is the sympathetic nervous system. Only a small fraction may be derived from the adrenal medulla.

3. Excessive urinary excretion of epinephrine and/or nor-epinephrine in pheochromocytoma could be demonstrated in cases with persistent hypertension as well as in cases of paroxysmal hypertension during or between attacks.

4. The methods used consisted in part of adsorption of the urinary catecholamines on precipitated aluminum hydroxide, followed by elution, desalting and concentration in vacuo. These extracts were studied by bio-assay, paper chromatography, photofluorometric evaluation and absolute quantitation of nor-epinephrine and epinephrine by chemical methods.

5. Photofluorometric evaluation of urine extracts seems to constitute the most reliable test for pheochromocytoma.

6. A short procedure, consisting of adsorption of catecholamines in hydrolyzed urine on an aluminum oxide column, followed by photofluorometric evaluation of the eluate, can be used as a rough screening test. When positive results are obtained, the longer and more specific method has to be applied for confirmation.

Addendum: After completion of the manuscript an additional group of forty-four cases was studied, who were suspected of pheochromocytoma because of clinical findings or positive pharmacologic tests.

The short screening procedure (method *2*) gave one false positive. In this case the long screening procedure (method *1c*) gave a negative result.

In eight patients of this group false positive responses to regitine were obtained and in three false positive responses to histamine. Six pheochromocytomas were detected. The urines of all the six cases of pheochromocytoma showed a B-1/B-2 ratio between 0.7 and 2.0, when the long method (*1c*) was used. However, with the short procedure (method *2*) a ratio of 3.0 was observed in one case.

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Quantitative Evaluation of Primary Adrenal Cortical Deficiency in Man*

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The past fifteen years have witnessed the development of a multiplicity of methods for the detection of clinical adrenal cortical deficiency. Screening tests of appealing simplicity[1–3] have been introduced, and more elaborate definitive methods[4,5] have invested the diagnosis of adrenal cortical inadequacy with a high degree of reliability. Methods have likewise been proposed to distinguish between primary failure of the adrenal cortex and atrophy secondary to disease of the adenohypophysis,[5] and to evaluate selective derangements of the several functions of the adrenal cortex.[6]

Although the magnitude of adrenal cortical reserve can be estimated by measuring the increment of urinary 17-ketosteroid following intravenous administration of corticotrophin,[5] no method for quantifying with precision the entire range of primary adrenocortical deficiency has, to our knowledge, been developed for clinical use, nor, indeed, in the physiologic laboratory. The development of subtotal adrenalectomy as an experimental therapeutic procedure in severe hypertensive vascular disease[7–13] has made the development of a precise method for quantifying adrenal function a matter of immediate practical concern as well as theoretic interest.

In view of the evidence presented by Sayers[14] and others, indicating that the secretion of corticotrophin by the adenophypophysis is stimulated by reduction in the titer of adrenal hormones or their metabolites in the body fluids, it appeared probable in advance that a small adrenal cortical remnant incapable of secreting a physiologically adequate quantity of hormone would be at all times under very nearly maximal stimulation by endogenous corticotrophin, so that little effect of exogenous corticotrophin would be detectable. Accordingly, it seemed likely that while a positive response to administered corticotrophin would permit one to infer the presence of sufficient adrenal tissue to provide some degree of functional reserve, lesser amounts of adrenal function might well be still further separable into several grades, corresponding anatomically to (1) a small adrenal remnant, constantly under near maximal stimulation and secreting inadequate or scarcely adequate quantities of hormone; and (2) a vanishingly small quantity of functioning adrenal cortical tissue. Since no consequential response to exogenous corticotrophin would be anticipated in either of these instances, the distinction between them would have to be made by observing the physiologic consequences of the institution of a standard regimen designed to provoke acute adrenal insufficiency in the adrenal-deficient subject.‡ Such a regimen or provocation test would consist of a period of

‡ There is need in endocrinology for two terms to express two distinct concepts: (1) lack of functioning endocrine tissue, and (2) physiologic consequences attributable to such lack. In this communication, for want of better available terminology, we understand by adrenal deficiency lack of adrenal cortical tissue, and by adrenal insufficiency, symptomatic and physiologic manifestations attributable to inadequacy of adrenal cortical hormone.

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† Trainee of the National Heart Institute.

observation on a metabolic ward during which exogenous hormone would be withdrawn and a standard constant diet of rather low sodium content provided.

Our experience with a combined provocation and corticotrophin test in a group of patients previously subjected to therapeutic subtotal adrenalectomy has encouraged us to suppose that it is in fact possible to classify adrenal functional capacity into four distinct categories. In general, it has been found that certain of these adrenal-deficient patients develop severe adrenal insufficiency during the provocation test; others exhibit symptomatic and chemical evidence of acute adrenal insufficiency of milder degree, yet show little evidence of responding to powerful corticotrophin stimulation; while patients in a third group manifest no signs of adrenal insufficiency during the provocation test, and respond to corticotrophin stimulation, although their response is quantitatively subnormal. To these respective types of behavior have been assigned the arbitrary grade numbers 0, 1 and 2, which are taken to correspond to three subdivisions, as indicated in Figure 1, of a continuum of degrees of adrenocortical deficiency. Grade 3 is reserved for adrenocortical adequacy with quantitatively normal functional reserve.

The observations upon which these generalizations are based will now be presented in detail.

METHODS

Clinical Material. The subjects studied comprised nine patients who had previously undergone subtotal adrenalectomy because of severe hypertensive vascular disease. All but two had also been subjected to prior sympathectomy and splanchnicectomy. Clinical data concerning these patients are presented in Table I. In addition, four healthy volunteers and two ambulatory patients who were in no way incapacitated or debilitated were studied as representatives of the normal corticotrophin response.

Metabolic Balance Studies. Adrenal-deficient patients were admitted for a period of seven days to the Metabolic Unit, and during this time metabolic balance studies were carried out. The total hospitalization was subdivided into a control period of three days plus an experimental period of four days, on the last of which an intravenous infusion of 10 international units of corticotrophin* was administered intravenously in 1 L. of 5 per cent aqueous dextrose over the first eight hours of the twenty-four-hour period.

The diet of each patient was kept constant throughout his stay, save for a supplement, in most of the patients, of sodium chloride during the control period. (Table I.) Each patient continued, during the control period, to receive his usual minimum maintenance steroid therapy, if any. During the subsequent experimental period, all steroid therapy was withheld, and the composition of the diet during this period was the same for all patients with respect to sodium and potassium (approximately 110 and 77 mEq., respectively) save for minor adjustments made in deference to the patient's food preferences. The caloric value of the diets was also standard (1,800

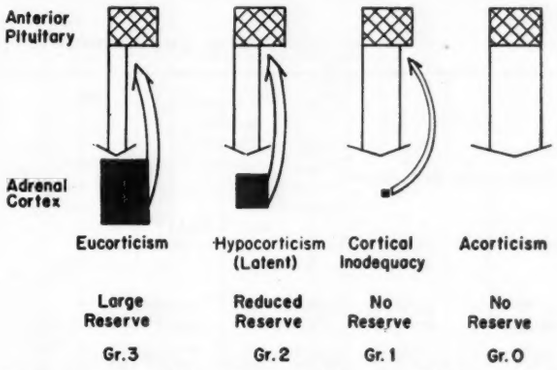

FIG. 1. Effect (schematic) of adrenal ablation upon secretory activity of adrenal cortex and adenohypophysis. The diagrams are predicated upon the assumption that homeostasis with respect to the titer of adrenocortical hormone (or its metabolites) in body fluids is maintained by means of the cybernetic mechanism described by Sayers.[14] From left to right are shown successive physiologic consequences which follow, on this assumption, as progressively increasing primary reduction of the quantity of adrenal cortical secretory tissue is surgically effected. In this special case of primary adrenocortical deficit, the homeostatic maintenance of a normal titer of cortical hormone (or its metabolites) in body fluids is tantamount to maintenance of a constant, quantitatively normal basal rate of secretion of cortical hormone. The rate of cortical hormone secretion is indicated by the width of the arrow from adrenal to pituitary, and the rate of corticotrophin secretion by the width of the arrow from pituitary to adrenal. The rate of secretion of cortical hormone remains practically constant, in spite of the decreasing mass of adrenal tissue, until it can no longer be maintained even by maximal stimulation of the adrenal by endogenous corticotrophin. Beyond this point, exogenous corticotrophin would not be expected to afford a means of distinguishing degrees of adrenocortical inadequacy.

* Dr. E. C. Vonder Heide, of Parke, Davis and Co., kindly supplied the corticotrophin used in this investigation.

to 2,000 calories per day), as was fluid intake (2.2 to 3.0 L. daily).

The meats, canned vegetables and fruit juices from which the diets were constructed were purchased in uniform lots. The mineral and nitrogen content of the lots was ascertained as the mean of multiple analyses of each foodstuff, from which the composition of the diets was then calculated.

Daily twenty-four-hour collections of urine were made, and any vomitus was saved for analysis. The mean daily stool values for the three-day control period and for the four-day experimental period were used in calculating the twenty-four-hour balances for M. H., T. S., B. L. and E. M. The potassium balances presented in the tables are uncorrected for nitrogen balance.[15]

The urine and stool collections were analyzed for sodium, chloride, potassium and nitrogen, and the urines also for creatinine. Daily venous blood samples were drawn for analysis of serum sodium, chloride, potassium, carbon dioxide content, protein, and whole blood or serum urea nitrogen, and for whole blood glucose, hemoglobin and hematocrit. Urinary steroid excretion was also studied three times, on the third, fifth, or sixth, and seventh day of the hospital stay.

Analytical Methods. Sodium and potassium were determined with the aid of a flame photometer equipped with internal lithium standard.[16] The following methods were used for the other chemical analyses:* chloride, Eisenman's modification[17] of the Van Slyke method: urea, Karr;[18] urine nitrogen, and in some individuals serum protein, by a micro-Kjeldahl method using a selenium catalyst and boric acid titration; serum protein in other individuals by the biuret reaction;[19] creatinine, Bonsnes and Taussky;[20] neutral reducing lipids (corticoids) after acid (pH 1) hydrolysis and after enzymatic (β-glucuronidase) hydrolysis of urine, Heard, Sobel and Venning;[21] neutral 17-ketosteroids, Holtorff and Koch.[22]

TABLE I

CLINICAL AND EXPERIMENTAL DATA: ADRENALECTOMIZED PATIENTS

Patient	Age	Sex	Race	Surface Area (m^2)	Adrenal Resection (Surgical Estimates)		Postop. Period (mos.)	Prior Sympathectomy	Pigmentation	Daily Dietary Sodium and Replacement Therapy during Control Period			Inulin Clearance ± S.E.M.	
					Left (%)	Right (%)				DCA (mg.-buccal)	Cortisone (mg.-oral)	Na (mEq.)	Control (ml./min.)	Provocation (ml./min.)
H. L.	51	M	W	1.80	90	90	9	0	+	0	9	116	42 and 36	40.3 ± 5.5
M. H.	31	F	W	1.65	97	100	5	SD*	0	0	37.5	249	64.1 ± 5.3	47.8 ± 7.2
T. S.	37	M	W	2.04	95	100	3	TL†	0	4	25	253	X	X
S. W.	47	M	C	1.98	90	95	19	0	+	2	25	285	X	74.3 ± 16.9
J. C.	41	F	W	1.47	90	100	5	SD*	0	2	37.5	163	50.0 ± 1.7	45.3 ± 2.0
B. G.	44	F	W	1.52	95	100	10	SD*	0	0	8	118	98.9 ± 24.1	100.0 ± 2.7
M. M.	49	F	W	1.75	95	100	11	SD*	0	0	4	118	97.0 ± 7.7	88.0 ± .92
B. L.	18	F	W	1.42	95	100	14	SD*	0	0	0	258	X	X
E. M.	37	M	W	2.01	90	100	17	SD*	0	0	0	251	X	X

* SD—Subdiaphragmatic sympathectomy.
† TL—Thoracolumbar sympathectomy.

RESULTS

Basic data obtained in the study of patients and volunteers are presented in Tables I, III, IV and V; derived data and their application as interpretive criteria in Tables II through V.

General survey of the data shows clearly, as would be expected according to the hypothesis illustrated in Figure 1, that the provocation test and the corticotrophin test collaborate in demarcating the two lower from the two higher grades; for the response to corticotrophin has been inconspicuous in patients who, during the provocation period, developed symptomatic and chemical evidence of acute adrenal insufficiency, but quite definite in those individuals who remained asymptomatic following hormone withdrawal. (Table II.) Evaluation of response to either portion of the test obviously presupposes the use of particular criteria of response. In Table II are presented the criteria which have been adopted for grading purposes on the basis

* We are indebted to Drs. J. R. Elkinton and John G. Reinhold for the performance of some of the analyses in their laboratories.

of a review of all the data obtained in the nine adrenalectomized patients and in the volunteers. Responses to each portion of the test characteristic of each grade of adrenal functional capacity are also indicated in semiquantitative fashion in Table II and are depicted schematically in Figure 2.

differences in response to the provocation test, while the corticotrophin test bears the entire burden of distinguishing grade 2 from the normal (grade 3).

It has been necessary to implement the semiquantitative expressions of Table II by defining the responses characteristic of each grade in

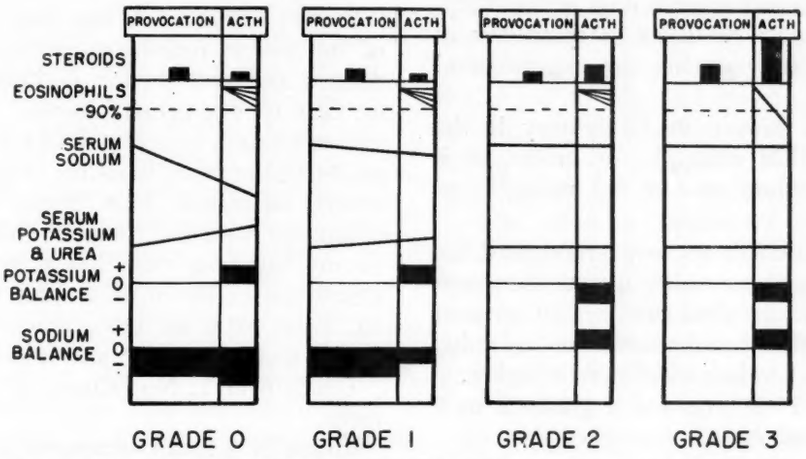

FIG. 2. Schematic representation of typical responses to provocation and corticotrophin test for each grade of adrenal function.

It is evident that there is some variation in the range and sensitivity of different indices of response to each portion of the test; but all indices which appear to be useful and reliable have been employed for grading in order to obtain maximum accuracy. The grading system indicated in Table II and Figure 2 accordingly represents a schema based upon recognized principles of adrenal physiology as confirmed in man by experience with a variety of indices of response. As shown in Table II, the distinction between grade 0 and grade 1 rests chiefly upon terms of exact mathematical limits. Such limits, again, have been erected in the light of a review of our experience to date; they are open to revision, for too few cases have been studied to permit much confidence that the exact present limits will necessarily remain the most serviceable ones. Limits at present proposed to quantify the criteria of Table II are as follows:

Provocation Test

Symptoms and Signs. Grade 0: Orthostatic faintness, usually with tachycardia and hyperp-

TABLE II

CRITERIA FOR QUANTITATIVE EVALUATION OF ADRENOCORTICAL FUNCTION*

	Provocation Test			Corticotrophin Test		
	Symptoms	Serum Values	Electrolyte Balances	Electrolyte Balances	Urinary Steroids	Eosinophils
Grade 0	++ (Progressive increase)	++	+	0	0	Non-significant
Grade 1	+	+	+	+	0	Non-significant
Grade 2	0	0	0	++	+	Non-significant
Grade 3	(0)†	(0)†	(0)†	++	+++	Decrease 90% or more

* + indicates positive response, i.e., evidence of adrenal insufficiency during the provocation test, or of available adrenocortical reserve during the corticotrophin test.

† Assumed; no observations made of effect of provocation test in normal volunteers.

nea, and lassitude, weakness, anorexia, nausea, or vomiting, becoming increasingly severe throughout the experimental period.

Grade 1: The above symptoms becoming manifest during the first twenty-four to forty-eight hours of the provocation period, but without further progression, or with actual amelioration, on the sixth and seventh days of the study.

Grade "2 or 3": No signs or symptoms of adrenal insufficiency during the experimental period.

Serum Values. Grade 0: Obligatory if the serum falls to 128 mEq./L. or below, or if the serum potassium rises to 6.2 mEq./L. or more.

Grade 1: Grade 0 is not warranted, and the serum urea increases steadily during the provocation period to the total extent of 20 per cent as compared with either the mean control value or the last control value, whichever is higher.

Grade "2 or 3" is recorded if grades 0 or 1 are not warranted.

Electrolyte Balances

Sodium. Greater sensitivity for grading purposes is attained by considering the sodium balances of the two portions of the test in conjunction with each other, as follows:

Grade 0: Negative mean daily balance during the provocation test with a more negative balance during the corticotrophin test.

Grade 1: Mean daily balance negative during the provocation test with balance during the corticotrophin test negative, but less so than during the provocation test.

Grade "2 or 3": Balance positive during the corticotrophin test.

When florid adrenal crisis requiring interruption of the test is attended by grade 0 change of serum electrolytes, the final grade 0 is *ipso facto* established; the corticotrophin test, which under these circumstances cannot be obtained at the scheduled time, is not needed for grading. If the corticotrophin test is not obtained, for this or any other reason, a negative mean daily balance of sodium of 40 mEq. or more during the provocation period may be graded "0 or 1."

Potassium. Grade "0 or 1": Positive balance during the corticotrophin test; mean daily balance during provocation test +10 mEq. or more.

Grade "2 or 3": Negative balance during the corticotrophin test; mean daily balance during provocation test less than +10 mEq.

Corticotrophin Test

Eosinophil Count. Grade "0, 1, or 2": An eosinophil decrease of less than 90 per cent at the end of the corticotrophin infusion.

Grade 3: A decrease of circulating eosinophils of 90 per cent or more at the end of the corticotrophin infusion.

Urinary Steroids. A base-line value (average of the twenty-four-hour excretion during the control and provocation periods) is calculated for each type of urinary steroid. These base-line values for each type of steroid are then averaged to obtain the mean base-line value of *all* types of steroid measured. This "mean" (Table IV) is compared with the "mean" (Table IV) of the twenty-four-hour excretion of the three types of steroid during the corticotrophin test; the change (Δ, Table IV) is an index of adrenal responsiveness to corticotrophin* and is graded as follows:

Grade 0 or 1: No change, or a mean decrement.

Grade 2: A mean increment of 4.0 mg. or less per twenty-four hours.

Grade 3: A mean increment greater than 4.0 mg. per twenty-four hours.

I. SELECTION AND PRECISE DEFINITION OF INTERPRETIVE CRITERIA

Provocation Test. During the provocation period symptoms and chemical alterations of the blood serum attributable to adrenal insufficiency

* It is recognized that the two "means" (pre-ACTH and post-ACTH) of steroid excretion shown in Table IV do not themselves possess any clear biological meaning. However, the mean change (Δ) which is used for grading is mathematically equivalent to the average change in urinary excretion of three types of steroids in response to corticotrophin stimulation. Since the change of any one of these types of steroid in response to corticotrophin is itself an acceptable index of adrenal stimulation, it is quite permissible to utilize an average value as a derived index; and because in the adrenal-deficient patients the changes are small compared with the random variability of the determinations, an obvious gain in reliability is achieved by the use of this derived index. One could with equal propriety use the total increment for the three steroid types as the derived index of adrenal responsiveness. The mean was chosen for present purposes because in two instances (J. C. and B. G.) corticoid determinations after glucuronidase hydrolysis of the urine were not done. The mean steroid increment in these patients is, of course, not strictly comparable with the values for the other patients; but it may be noted that omission of the values for this steroid type from the calculation of the mean in each of the other adrenal-deficient patients would cause a change in grade in only one instance (B. L.).

have been confined to patients graded 0 or 1. The failure of such alterations to appear in the case of grade 2 patients presumably indicates the existence of adrenal cortical reserve sufficient to maintain homeostasis in the face of the mild stress afforded by reduction of dietary sodium for four days.

Symptoms and signs (*Table* v)*:* The symptoms associated with postural hypotension (faintness, tachycardia and hyperpnea on quiet standing) have been the earliest and the most sensitive indicator of mild adrenal insufficiency in most of these patients. When a prior subdiaphragmatic sympathectomy had been performed, the occurrence of postural hypotension and its attendant symptoms cannot be ascribed exclusively to adrenal insufficiency. While these symptoms have been very useful indicators of incipience and progress of acute adrenal insufficiency in our patients, they might well be less helpful in adrenal-deficient patients with intact peripheral sympathetic nerves, although postural hypotension is of common occurrence in Addison's disease and was a prominent feature of adrenal insufficiency in H. L. The other symptoms noted were lassitude, weakness, anorexia, nausea and (in H. L.) vomiting. The supine blood pressure often declined but this variable has not proved to be useful for grading purposes.

Symptoms are not readily quantified, but there has been little difficulty in recognizing the symptomatic response to the provocation test which characteristically appears in patients graded 0 or 1. Patients graded 2 have developed no symptoms during the provocation period. Grade 0 has been distinguished by the *progression* of the symptoms, for such patients have felt steadily worse after hormone withdrawal. Grade 1 patients, by contrast, have generally experienced more severe symptoms during the first two days of hormone withdrawal than during the last two days of the experimental period.

Serum sodium, potassium and urea (*Table* iii)*:* There has been a clear-cut distinction between grade 0 patients and all others with respect to the blood chemical findings. Serum sodium concentration fell well beyond the lower limit of normal (132 mEq./L.) in H. L. and M. H. but remained within normal limits in all other patients. Serum potassium concentration rose higher in these patients than in any others but abnormal elevation of serum potassium also occurred in grade 1 patients.

Some caution may perhaps be in order in applying generally the quantitative limits based upon the observed rise of serum urea and potassium in these patients. Such measurements of glomerular filtration rate as were made (Table i) suggest that this function was somewhat more impaired in these patients (though not greatly so) than it is in Addison's disease.[23,24] The effect of an independent cause for reduction of glomerular filtration would be to elevate the base-line serum urea concentration during the control period and to augment the absolute rise resulting from any standard decrease of filtration rate during the experimental period. Expression of observed change as a percentile increase will, however, tend to compensate for any effect of coexisting renal disease upon the quantitative estimate. There may be some reason to suspect[25] that the magnitude of the rise of serum potassium in acute adrenal insufficiency is also increased in the presence of concomitant chronic renal disease.

Electrolyte Balances (*Table* iii). The interpretation of sodium balance appears to give more precise information when the data from the provocation period are considered in conjunction with the balance on the day of administration of corticotrophin. The potassium balance during the provocation period was always more positive in the patients with more severe adrenal deficiency; a mean balance of potassium during the provocation period of +10 mEq. or more may therefore be graded 0 or 1. Likewise, simple determination of the sign of the potassium balance during the corticotrophin test has permitted two higher grades to be distinguished from the two lower in all but one instance.

Corticotrophin Test (*Table* iv). In addition to the electrolyte balances considered above, the eosinophil count and the urinary excretion of steroids have been used as measures of adrenal stimulation by corticotrophin.

Eosinophil count: Normal volunteers all showed a reduction of circulating eosinophils of more than 90 per cent at the termination of the eight-hour infusion. All adrenal-deficient patients exhibited a smaller decrease, but the magnitudes of decreases less than 90 per cent fail to correlate with the other indices of adrenal deficiency.

Urinary steroids: The data indicate that when steroid therapy is withdrawn the urinary steroid elimination tends to decrease during the provocation period whereas in patients not receiving steroids during the control period no consistent change occurs. If the mean of the control and

provocation period steroid excretion is used as the basis for computing the increase following corticotrophin stimulation, a sharp separation of grades 1 and 2 is obtained. (Table IV.) There may be some theoretical objection to using the mean value as a control, on the ground that the possessing normal adrenal reserve indicate that our grade 2 patients have markedly subnormal adrenocortical reserve as judged by this criterion. The hiatus between the small responses observed in grade 2 patients and the large mean increments in normal individuals (Table IV) suggest

TABLE III

METABOLIC DATA—ADRENAL-DEFICIENT PATIENTS

Metabolite	Serum Concentrations* Day of Experiment 1	2	3 (Control Period)	4	5	6 (Provocation Period)	7 (ACTH)	Grade (Provocation Test)	Patient	Electrolyte Balances† Day of Experiment 1	2	3 (Control Period)	4	5	6 (Provocation Period)	7 (ACTH)	Mean Daily Balance During Provocation Period	Grade (Balances)
									H. L.									
Na	137	134	132	123	121	117	115			−56	−38	−32	−21	−16	−23	−57	−20	0
K	5.2	5.2	5.5	5.6	5.5	5.5	6.3	0		+13	+12	−2	+12	+18	+8	−27	+13	1+
SUN	35	X	43	46	45	43	50											
									M. H.									
Na	132	136	138	134	130	123‡	X			+96	+33	−14	−84	−21	X	X	−53	0 or 1
K	5.0	5.4	5.3	5.0	5.4	6.2‡	X	0		+37	+11	−6	+24	+1	X	X	+12	0 or 1
SUN	34	33	30	33	32	30‡	X											
									T. S.									
Na	141	142	141	143	140	141	139			−188	−12	+23	−113	−47	−8	−27	−56	1
K	4.7	5.0	4.9	5.6	5.4	5.6	5.9	1		−7	+13	+11	+21	+19	+20	+10	+20	0 or 1
SUN	30	31	33	36	43	45	49											
									S. W.									
Na	139	146	141	138	138	137	137			+69	+15	−25	−121	−62	−76	−18	−86	1
K	5.4	5.3	5.0	5.4	5.7	5.9	6.1	1		+5	+15	−3	+25	+15	+23	+11	+21	0 or 1
SUN	37	36	36	37	42	47	56											
									J. C.									
Na	139	142	143	140	138	136	137			−15	−58	−56	−39	−44	−45	−18	−43	1
K	4.1	4.2	4.4	4.4	4.2	4.5	4.7	1		+20	+8	−19	+19	+18	−1	+1	+12	0 or 1
SUN	27	32	34	37	41	X	41											
									B. G.									
Na	138	135	134	135	137	146	139			−7	−30	−7	−11	−4	−30	−12	−15	1
K	4.5	4.8	4.6	5.2	4.6	4.7	4.3	2 or 3		+30	+18	+13	+17	−17	−19	−9	−6	2 or 3
SUN	19	19	22	22	22	23	21											
									M. M.									
Na	139	137	135	139	134	137	134			−59	−44	−65	−20	−7	−22	+18	−16	2 or 3
K	4.7	4.7	4.8	5.3	5.1	5.1	4.8	2 or 3		+21	+7	+19	+1	+3	+6	−70	+3	2 or 3
SUN	23	X	23	21	26	28	25											
									B. L.									
Na	138	136	141	137	138	137	138			+30	−10	+43	−36	−16	−5	+27	−19	2 or 3
K	3.9	3.8	3.9	3.8	3.9	3.8	3.9	2 or 3		+3	+1	+8	+15	+7	+4	−3	+9	2 or 3
SUN	17	15	14	21	21	22	21											
									E. M.									
Na	144	141	142	142	144	144	145			+6	−9	+50	−70	−34	−2	+18	−35	2 or 3
K	5.1	X	5.1	4.9	4.7	4.9	4.8	2 or 3		−1	−2	+5	+4	+8	+13	−4	+8	2 or 3
SUN	27	24	24	23	25	26	23											

* Values for end of each listed twenty-four-hour period. Serum urea nitrogen (SUN) calculated in three cases (H. L., B. G., M. M.) from BUN; measured in all other patients. SUN in mg. per 100 cc.; Na and K in mEq./L.

† Balances in mEq. per twenty-four hour.

‡ Values after twelve hours of sixth day period.

steroid replacement therapy is an extraneous factor contributing to the estimation. On the other hand, the use of a mean control value undoubtedly promotes accuracy by decreasing the random variability of the determination, and this practice has accordingly been adopted.

The distinction between grade 2 and grade 3 rests wholly upon the corticotrophin test. The urinary steroid increments observed in response to corticotrophin in individuals presumably that an intermediate grade of subnormal response could be interpolated between grade 2 and 3. If the normal range of response is defined as the mean increment observed in the volunteers plus or minus two standard deviations, the normal increment will be 6.0 to 10.9 mg. This leaves a gap between the largest grade 2 response, a 1.0 mg. mean increment, and the lower normal of 6.0 mg.; and it is noteworthy that the one patient studied following unilateral adrenal-

TABLE IV

CHANGE IN URINARY STEROID EXCRETION (MG. PER 24 HOURS) AND CIRCULATING EOSINOPHILS (PER CU. MM.) IN RESPONSE TO CORTICOTROPHIN TEST

Adrenalectomy Patients

Names	Period	Corticoids		17-KS	Mean*	Δ	Grade	Eosinophil Count¶	Δ%	Grade
		pH 1‖	β-glucuronidase††							
H. L.	Control	2.1	X	10.2						
	Provocative	2.0	5.5	X	5.89		0 or 1	419		
	ACTH	1.2	3.3	2.9	2.47	− 3.42		231	−45	<3
T. S.	Control	2.4	10.5	8.1						
	Provo.	2.4	6.2	6.0	5.93			153		
	ACTH	2.1	7.5	6.5	5.37	− .56	0 or 1	51	−67	<3
S. W.	Control	1.9	7.5	7.6						
	Provo.	2.3	3.6	2.5	4.23			213		
	ACTH	1.1	3.2	2.7	2.33	− 1.90	0 or 1	76	−64	<3
J. C.	Control	2.3	X	5.3						
	Provo.	1.4	X	1.8	2.70			138		
	ACTH	1.9	X	1.5	1.70	− 1.00	0 or 1	103	−25	<3
B. G.	Control	1.9	X	4.0						
	Provo.	2.8	X	5.5	3.55			320		
	ACTH	3.0	X	6.0	4.50	+ .95	2	205	−36	<3
M. M.	Control	1.8	X	5.4						
	Provo.	2.3	9.2	8.4	6.05			138		
	ACTH	2.5	8.9	8.4	6.60	+ .55	2	125	−9	<3
B. L.	Control	3.6	7.5	7.5						
	Provo.	3.2	9.9	7.3	6.50			259		
	ACTH	3.4	12.0	6.2	7.20	+ .70	2	34	−87	<3
E. M.	Control	3.6	21.4	13.1						
	Provo.	2.7	X	16.2	13.07			100		
	ACTH	4.1	22.4	15.8	14.10	+ 1.03	2	69	−31	<3
					Volunteers					
G. W.	Control	7.6	16.4	26.6						
(M)	Control	7.2	15.0	27.2	16.67			229		
	ACTH	9.9	30.4	32.0	24.10	+ 7.43	3	6	−97	3
G. H.	Control	5.8	12.6	16.1						
(M)	Control	5.4	16.2	10.0	11.02			210		
	ACTH	7.0	25.7	26.2	19.63	+ 8.61	3	12	−94	3
J. M.	Control	3.7	10.6	20.9						
(F)	Control	4.6	11.3	14.6	10.95			470		
	ACTH	8.7	25.2	29.0	20.97	+10.02	3	4	−99	3
H. B.	Control	3.6	11.0	8.6						
(F)	Control	3.9	7.8	8.2	7.18			99		
	ACTH**	5.6	17.4	22.4	12.10	+ 7.38**	3	3	−97	3
D. K.†	Control	2.9	10.0	12.9						
(M)	Control	3.7	X	16.0	9.25			55		
	ACTH	5.1	25.2	26.8	19.03	+ 9.78	3	0	−100	3
C. M.‡	Control	4.5	11.7	19.4						
(F)	Control	3.7	8.7	16.5	10.75			58		
	ACTH	7.0	22.6	24.9	18.17	+ 7.42	3	3	−95	3
					Mean:	+ 8.44 ± 1.22				
C. W.§	Control	2.2	7.3	10.4						
(M)	Control	4.6	14.9	13.8	8.87			148		
	ACTH	3.4	17.1	23.1	14.53	+ 5.66		6	−96	3

* See text. Where only one pre-ACTH value for a steroid type was obtained, it was used as the estimate of the mean pre-ACTH value.

† Subacute glomerulonephritis.

‡ Hemorrhoids.

§ Prior unilateral adrenalectomy and sympathectomy for hypertension.

¶ Count at end of ACTH infusion appears below count prior to infusion.

‖ pH 1: Urine hydrolyzed with hydrochloric acid at pH 1.

†† Urine hydrolyzed at pH 4.5 with β-glucuronidase prior to acid hydrolysis.

** Six-hour corticotrophin infusion. Mean steroid increment $\times \frac{8}{6}$. See reference 26.

ectomy falls within these limits. However, because of the scantiness of the data and because this distinction would have to be based wholly on steroid response, we have not proposed an intermediate grade. Instead, the definition of a subnormal steroid response has been set arbitrarily at a point about the middle of the hiatus.

The changes following corticotrophin administration of circulating eosinophils and 17-ketosteroid excretion observed by us in a smaller number of normal individuals may be compared with the much larger series reported by Renold et al.[26] Other data presented by these authors show that either 20 units—the quantity employed in their studies—or 10 units constitute a virtually maximal stimulating dose when administered intravenously over eight hours; and there is satisfactory agreement of our data with theirs with respect to the normal response in terms of these indices to an eight-hour infusion. We know of no published figures concerning the normal augmentation of corticoid elimination in response to standard stimulation.

It is possible that the response of the electrolyte balances to corticotrophin is also quantitatively subnormal in grade 2 patients but the opportunity to establish normal limits of response in terms of this criterion has not presented itself.

II. CRITIQUE OF THE STUDIES AND OF THE METHOD

With respect to the design of the experiments, certain refinements, attractive on theoretical grounds, have been rejected for practical reasons. A longer control period, while manifestly desirable, seemed to us financially unjustifiable unless it could be shown to be an absolute necessity. No doubt also in theory the standard experimental diet might better have had its caloric value and sodium and potassium composition related to the body weight or estimated caloric requirement of the subject. Practically, the dietary management is greatly simplified by a reasonably uniform practice, which accordingly was adopted. Scheduled experiments have not been cancelled on the ground that recent cessation of the menses might complicate the interpretation of the electrolyte balances, nor were such extraneous influences, even when suspected, permitted to complicate further the criteria for interpreting the data. Indeed, we went so far as to administer small doses of cortisone* intravenously over approximately thirty minutes to B. G. and J. C. (10 and 24 mg., respectively) in conjunction with another investigative problem on the sixth day of the study. The results indicate that such deviations from ideal experimental practice have not seriously compromised the interpretation of the data for grading purposes.

The sodium and potassium content (110 and 77 mEq. per day, respectively) of the diet during the provocation period has proved to be satisfactory in that on such a diet severely adrenal-deficient individuals will develop frank acute adrenal insufficiency within the period allotted for observation of their response. A uniform practice was not adopted in all patients with regard to the dietary sodium of the control period; had this been done the sodium balance during the provocation period might have constituted by itself a more sensitive index of adrenal deficiency. There is no doubt that the adrenal cortex plays an important role in the adaptive mechanism by which the negative sodium balance which follows dietary salt withdrawal is minimized and ultimately arrested in the normal subject. Presumably a more sensitive appraisal of available cortical hormone will be given by placing this adaptive mechanism under stress; and accordingly we favor the provision of about 250 mEq. of sodium during the control period. The balance data on H. L., in whom no change of dietary sodium was effected in the beginning of the provocation period, suggest that under the latter conditions sodium wastage may fail to afford a satisfactory quantitative index of hormonal status.

The arbitrary character of certain of the mathematical definitions (e.g., the upper limit for serum sodium concentration as characteristic of grade 0) is in part the consequence of scanty data, but also in part inherent in the process of erecting boundaries within a continuum, an enterprise in which the location of any line of demarcation is essentially arbitrary and is governed by convenience. It is not, for example, implied by the use of the symbol 0 that a state of total adrenocortical deficiency exists—a proposition presumably not susceptible of conclusive demonstration either during life or postmortem by any practical means—but only that functional capacity is demonstrably less in such patients than in those graded 1.

As has been previously indicated, there are suggestions that the response of an individual

* Cortisone free alcohol in physiologic saline, kindly supplied by Dr. Elmer Alpert of Merck and Company.

to acute adrenal insufficiency may be modified by coexisting organic disease. Thus postural hypotension is probably a more prominent manifestation of acute adrenal insufficiency when sympathectomy has been performed and azotemia may be a more prominent feature of adrenal insufficiency when renal function is impaired. Evidently, therefore, one or another index of adrenal insufficiency might become misleading as a basis for quantitative evaluation of adrenal deficiency if some unsuspected abnormality of other endocrine or non-endocrine organs were present. While there is nothing in the data to suggest that hypertensive disease affects the symptomatic and metabolic response of the organism to acute adrenal insufficiency save perhaps through impairment of renal function, the possibility of other modifying influences should be kept in mind. Moreover, the method will not give an accurate evaluation of the degree of adrenal deficiency unless the study is carried out under basal conditions, i.e., in the absence of stressful influences which would increase the need for hormone. (Table v.)

While it is quite true that the definitions of the limits for each criterion characteristic of the respective grades have been tailored to fit these particular patients and may require revision in a larger series, the most reassuring indication that the method gives a reliable quantitative evaluation of adrenocortical function is found in the excellent internal consistency of the grades assigned to any patient on the basis of eleven separate indices of response to two fundamentally different types of test, as summarized in Table v. It is difficult to believe that such a congruence could possibly be realized in nine individual patients unless the indices reflected rather reliably the quantitative state of adrenal functional capacity. It is certainly to be expected that intermediate grades, such as that assigned to B. G., will be encountered. However, the only major discrepancy of a single index—the seventh day potassium balance in H. L.—is explicable in that the patient vomited on the last day of the test.

Anatomic confirmation of our quantitative reports of adrenal functional capacity has been forthcoming in two patients. Some months after H. L. had been graded 0 he died following three days of a mild febrile illness signalized by diarrhea and vomiting which, in spite of repeated previous warnings, he failed to report to any physician. At necropsy, performed by Dr. F. E. Haentze of the Roxborough Memorial Hospital, Philadelphia, no adrenal tissue was found. B. L., who was classified as grade 2, has recently been re-explored surgically and a viable, excessively large left adrenal remnant was found and removed. Its weight was 1.1 gm., representing 11 per cent of the total adrenal tissue resected, as compared with the desired figure of about 3 per cent.

TABLE V

SUMMARY OF GRADES BY SEPARATE CRITERIA AND FINAL GRADE OF ADRENAL-DEFICIENT PATIENTS

Patient	Provocation Test		Electrolyte Balances		Corticotrophin Test		Final Grade
	Symptoms	Serum Values	Na	K	Steroids	Eosinophils	
H. L.	0	0	0	1+	0 or 1	<3	0
M. H.	0	0	0 or 1	X	X	X	0
T. S.	1	1	1	0 or 1	0 or 1	<3	1
S. W.	1	1	1	0 or 1	0 or 1	<3	1
J. C.	1	1	1	0 or 1	0 or 1	<3	1
B. G.	1	2 or 3	1	2 or 3	2	<3	1+
M. M.	2 or 3	2 or 3	2 or 3	2 or 3	2	<3	2
B. L.	2 or 3	2 or 3	2 or 3	2 or 3	2	<3	2
E. M.	2 or 3	2 or 3	2 or 3	2 or 3	2	<3	2

On the other hand, there is very poor correlation of the grades herein reported with the surgeon's estimate of the size of the adrenal remnant spared at operation. We are not inclined to interpret this discrepancy as impugning the physiologic estimation but rather as a reflection of such factors as the difficulty of precise anatomic quantification at the operating table and the impossibility of evaluating the variable effects of compromised blood supply and scar tissue formation upon the viability of the remnant, and of possible regeneration of glandular tissue following surgery.

Although the characteristic Addisonian melanosis has thus far been encountered only in patients graded 0 or 1, no information known to us permits assurance as to the minimum impairment of adrenocortical adequacy which can occasion such pigmentation. It may well be that the grade of 2 is compatible with melanosis. Certainly it is not improbable that certain patients with the diagnosis of spontaneous Addison's disease, documented by pigmentation and the occurrence of adrenal crisis, might be graded 2 by our method; for the stress (dietary sodium reduction) to which patients are subjected during the provocation period is a very mild one compared with those infections and similar

stresses which are encountered by most individuals from time to time. Quantitative evaluation of adrenocortical function in Addison's disease might clarify this and perhaps other aspects of that condition.

Quantitative evaluation of adrenal function by our method is so elaborate an enterprise that it can be useful only as an investigative tool. It must also be emphatically stated that deliberate induction of acute adrenal insufficiency involves a very real potential hazard to the patient. No such study should be undertaken unless the responsible physicians are thoroughly conversant with the recognition and management of adrenal crisis. We have perhaps erred on the side of caution several times by terminating an experiment, at the cost of losing the opportunity for precise grading, on the basis of our clinical impression of the patient's condition, although the blood chemical findings were subsequently found not to be deranged; for Pearson et al.[27] have withdrawn all steroid from totally adrenalectomized patients for as long as five to eight days, apparently without mishap. However, the serum values of M. H. (Table III) afford instructive evidence of the rapidity with which serum sodium and potassium concentrations can attain alarming levels in a patient exhibiting little clinical evidence of a grave turn of events. The investigator cannot evade the assumption of full responsibility for protecting the patient while conducting such studies.

SUMMARY

1. A method designed to afford a quantitative appraisal of adrenal cortical functional capacity has been devised. It consists of a two-fold test: (1) a standard metabolic regimen capable of provoking acute adrenal insufficiency in the severely adrenal-deficient individual; and (2) powerful adrenal cortical stimulation by means of intravenous corticotrophin.

2. Classification of patients into one of four separate grades of adrenal cortical functional capacity is proposed on the basis of multiple criteria of response to each portion of the test. Intrinsic and extrinsic grounds for confidence in the accuracy of the method are presented.

Acknowledgments: These studies could not have been carried out had not Dr. J. Russell Elkinton and his staff organized, equipped and put into operation the Metabolic Unit of the University Hospital. Their kindness in extending to us its facilities, the cooperation of Miss Helen Merton and her nursing staff, and the indispensable services of Miss Marie Wackerman, who alone worked out and prepared all the diets, are most gratefully acknowledged.

Technical assistance was rendered by Miss Catherine Macatsoris, Mrs. Irmgaard Landolt, Mr. Walter Applin, Mrs. Elizabeth Songster and Mr. William Luetzel.

Replacement medication for post-adrenalectomy patients has been supplied *gratis*. Cortone® was kindly given to us by Drs. Elmer Alpert and Augustus Gibson of Merck and Company; percorten® by Dr. F. L. Mohr, of Ciba Pharmaceutical Products, Inc.; cortate® by Dr. R. W. Burlew of Schering Corporation; and aqueous adrenal cortex extract by Dr. H. F. Hailman, of the Upjohn Company.

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Evaluation of the "Cortisone Test" As a Diagnostic Aid in Differentiating Adrenal Hyperplasia from Adrenal Neoplasia*

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It has been demonstrated in experimental animals that one of the physiologic effects of cortisone is the inhibition of ACTH secretion by the adenohypophysis (literature reviewed by Ingle[1] and by Sayers[2]). A similar phenomenon has been shown to occur in the human by Perera and Ragan,[3] Sprague[4] and his collaborators, and by others. Atrophy of the adrenals is often encountered at postmortem examination of patients who had received large doses of cortisone over long periods of time.

Clinical application of this ACTH inhibiting effect has been made by Wilkins et al.,[5] Bartter et al.[6] and Jailer[7] who have treated congenital adrenal hyperplasia by the administration of exogenous cortisone. When these patients are on this regimen, the urinary 17-ketosteroids which are characteristically elevated in this syndrome consistently fall to levels which are approximately normal for the age and sex of the patient. Presumably the abnormal adrenal is "put to rest" as a result of inhibition of ACTH secretion by the pituitary due to the administration of cortisone. On the other hand a similar fall in the urinary 17-ketosteroids did not occur in two patients with adrenal virilism due to an adrenal cortical carcinoma when cortisone was administered.[8] Four cases of adrenal tumor, the nature of which was not specified, have been reported by Gardner and Migeon[9,10] with similar results. Venning[11] has also described three patients with adrenal cortical tumors to whom the administration of cortisone caused but an equivocal fall in only one.

Apparently the same differentiation between adrenal hyperplasia and tumor may be made in Cushing's syndrome. Jailer, Louchart, Gold and Knowlton[12] reported that the administration of cortisone to five patients with documented adrenal hyperplasia resulted in a fall in urinary 17-ketosteroids. This fall was not as great as one finds in adrenal virilism, probably due to the lower initial control values observed in Cushing's syndrome. One patient with adrenal carcinoma failed to respond with a fall in his urinary 17-ketosteroids when given adequate amounts of cortisone.

The purpose of this communication is to present our experience with the "cortisone test" in thirty-six patients with adrenal hyperfunction, both of the virilizing and Cushing types. In addition we are reviewing all the reported cases of these conditions in which the "cortisone test" was applied to ascertain whether it may be used clinically with any degree of accuracy to differentiate adrenal tumor from hyperplasia.

METHODS AND MATERIALS

The following types of patients were used in this study: (1) fourteen female pseudohermaphrodites with virilism, (2) six boys with macrogenitosomia praecox due to adrenal hyperplasia, (3) five patients with adrenal virilism secondary to tumor, three with carcinoma and two with adenoma, (4) six patients with Cushing's syndrome due to adrenal hyperplasia, (5) five cases of Cushing's syndrome due to adrenal cortical tumors; three were car-

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cinomas and two were adenomas. These patients were between the ages of three weeks and forty-six years.

After suitable control periods the patients received varying amounts of cortisone* either intramuscularly or by mouth, as indicated in Tables I to III. In addition two patients received hydrocortisone intravenously. In the latter patients the urine collections were in four-hour aliquots; in all others they were in twenty-four hour periods. We have adhered to the following routine in performing the "cortisone test": (1) Daily 17-ketosteroid determinations are performed on twenty-four-hour urine specimens for at least two to four days before institution of the test. (2) A dose of 100 mg. of cortisone twice a day is administered intramuscularly for five days. In children the dosage may be decreased depending upon the age of the child. (3) Daily urine collections are continued during the period of administration of the cortisone and for one to two days thereafter.

Urinary 17-ketosteroids were determined by the method of Holtorff and Koch.[13] All but four of the thirty-six patients were studied while in the hospital.

RESULTS

I. Adrenogenital Syndrome

Bilateral Adrenal Hyperplasia. As indicated in Table I the administration of cortisone to twenty patients with adrenal hyperplasia and virilism resulted in a significant fall in the urinary 17-ketosteroids from the elevated control levels. In most instances, when adequate amounts of cortisone were given, the 17-ketosteroid values fell to levels which one would expect for the age and sex of the patient.

Adrenal Cortical Tumor. In none of the five patients with adrenocortical tumor which manifested itself primarily in the virilizing syndrome was there a significant fall in the urinary 17-ketosteroids. The same results were obtained in the presence of either carcinoma or adenoma. (Table II.)

II. Cushing's Syndrome

Bilateral Adrenal Hyperplasia. Five of the six patients in this group exhibited a significant fall in the urinary 17-ketosteroids upon administration of 200 mg. of cortisone a day. As will be

* The cortisone and hydrocortisone used in this study were kindly supplied by Dr. Elmer Alpert of Merck, Inc.

TABLE I

ADRENOGENITAL SYNDROME DUE TO ADRENAL HYPERPLASIA

Patient	Age (yr.)	Control* 17-KS Excretion (mg./24 hr.)	Cortisone Dosage (mg.) and Route of Administration	17-KS† Excretion on Cortisone Regimen (mg./24 hr.)
A—Female Pseudohermaphrodites				
K. A.	14	51.4	100 i.m. × 5 d.‡	5.6
R. E.	11	20.3	100 i.m. × 4 d.	4.6
T. K.	13	30.8	200 i.m. × 5 d.	13.3
A. M.	16	79.1	200 i.m. × 5 d.‡	13.6
J. L.	15	34.9	50 i.m. × 4 d.	9.6
B. L.	3	8.96	25 i.m. × 4 d.	2.2
C. B.	17	28.8	200 i.m. × 4 d.	10.6
F. K.	8	23.4	75 p.o. × 6 d.	6.1
D. P.	18	61.1	100 i.m. × 5 d.	20.1
C. A.	17	63.9	50 p.o. × 11 d.	1.6
C. D.	1½	13.6	50 i.m. × 4 d.	4.7
J. B.	8	13.5	75 i.m. t.i.w. × 30 d.	2.3
J. P.	3 wk.	9.2	5 p.o. × 6 d.	3.95
S. F.	3	6.5	25 p.o. × 5 d.	3.0
B—Macrogenitosomia Praecox				
Re. W.	4	16.5	100 i.m. × 9 d.	2.5
Ra. W.	6	26.3	100 i.m. × 5 d.	5.3
E. B.	9	34.6	200 p.o. × 4 d.	6.5
A. L.	8	19.2	25 p.o. × 10 d	13.2
W. H.	4	19.4	100 p.o. × 4 d.	2.4
L. Y.	3	9.5	100 i.m. × 4 d.	3.7

* Values represent average of 17-KS excretion over approximately a one-week period.

† Values represent average of 17-KS excretion of last two or three days of cortisone administration.

‡ Received hydrocortisone.

d. = days.

(Data on fourteen of these patients have been presented previously.[7b])

noted from Table III the response obtained in the males was not as great as that in the females. This is understandable since the testes are a source of a fraction of the 17-ketosteroid precursors and presumably are not inhibited by exogenous cortisone administration. However, in one patient (J. T., a female) the cortisone test was negative on two separate occasions both when the cortisone was given intramuscularly and also when hydrocortisone was administered intravenously. Histologically, the adrenal glands of this patient suggested small foam cell cortical adenomas in the presence of predominant cortical hyperplasia. This patient perhaps should be placed in the group of adrenal cortical adenomas and not hyperplasia.*

Tumor. In none of the five patients with adrenal tumors was there a significant fall in urinary 17-ketosteroids as a result of cortisone administration. It should be borne in mind, however, that two of the patients had low 17-ketosteroids during the control period and that under these circumstances, due to the admittedly crude methods of analysis, a detectable fall may not be observed.

TABLE II

ADRENOGENITAL SYNDROME DUE TO ADRENAL NEOPLASIA

	A—Adrenal Adenoma		B—Adrenal Carcinoma		
Patient	P. B. ♀	A. F. ♀	A. P. ♀	M. C. ♀	E. L. ♀
Age (yr.)	23	4½	13	42	20
Control 17-KS Excretion mg./24 hr.	56.0	14.9	36.5	24.0	(See Figure 1)
Cortisone Dosage (mg.) and Route of Administration	200 i.m. × 5 days	35 i.m. × 4 days	100 i.m. × 5 days	100 i.m. ×6 days	.....
17-KS Excretion on Cortisone mg./24 hr.	74.0	14.3	36.2	20.0	.....

TABLE III

CUSHING'S SYNDROME

	A—Adrenal Hyperplasia						B—Adrenal Adenoma		C—Adrenal Carcinoma		
Patient and Age (yr.)	S. K. ♂ 22	A. G. ♀ 30	H. H. ♀ 38	J. T. ♀ 41	C. C. ♀ 29	J. J. ♂ 31	M. T. ♀ 41	G. M. ♀ 36	M. S. ♀ 35	C. R. ♂ 26	K. S. ♀ 46
Control 17-KS Excretion mg./24 hr.	19.4	20.6	21.7	26.2	11.7	27.6	22.4	5.7	67.6	43.9	6.68
Cortisone Dosage (mg.) and Route of Administration	200 i.m. × 5 d.	200 i.m. × 4 d.	200 i.m. × 5 d.	200 i.m. × 5 d.	200 i.m. × 4 d.	200 i.m. × 5 d.	200 i.m. × 5 d.	200 i.m. × 3 d.	200 i.m. × 4 d.	100 i.m. × 4 d.	200 i.m. × 5 d.
17-KS Excretion on Cortisone mg./24 hr.	6.6	9.7	12.5	26.5	4.7	19.2	32.2	5.0	59.0	63.0	7.86

III. Intravenous Hydrocortisone Test

In an attempt to ascertain how quickly the fall in urinary 17-ketosteroids occurs an infusion of 100 mg. of hydrocortisone-alcohol was given to two patients over a four-hour period. After suitable four-hour control periods the infusion was administered over a four-hour period, with continued collection of urine in four-hour units. The results are shown in Figure 1. The one patient with adrenal virilism due to adrenal hyperplasia manifested a marked fall in 17-ketosteroids starting in the four-hour period immediately following cessation of the infusion. These values remained low for twelve hours and then rose to the pre-treatment levels. In the patient with adrenogenital syndrome due to adrenal carcinoma no such fall occurred, instead a rise was noted.

* The following report was submitted by Dr. M. M. Melicow, pathologist to the Squier Urological Clinic: "There is no evidence of a neoplasm in the usual sense in either specimen. The presence of the microscopic spheroids suggests the possibility of a beginning neoplasm. A diagnosis of such a process, though suspected, is, however, not possible."

COMMENTS

Apparently, hyperplastic adrenal glands in patients with adrenal virilism respond differently to the administration of cortisone and hydrocortisone than adrenal tumors. As can be seen in Table IVA and B, thirty-nine patients with

TABLE IV
REVIEW OF LITERATURE

	No. of Cases Reported	17-KS Fall with Cortisone
A—Congenital adrenal virilism—adrenal hyperplasia		
Bartter et al.[6]	2	Yes
Bastenie et al.[14]	1	Yes
Kelley et al.[15]	4	Yes
LaRoche et al.[16]	1	Yes
Mason et al.[17]	1	Yes
Shepard et al.[18]	1	Yes
Wilkins et al.[5,19–27]	19	Yes
Gastineau et al.[28]	1	Yes
Prader[29]	1	Yes
B—Acquired adrenal virilism—adrenal hyperplasia		
Bastenie et al.[14]	5	Yes
Venning et al.[11]	1	Equivocal
Gardner et al.[10]	1	Yes
Kupperman et al.[30]	1	Yes
C—Acquired adrenal virilism—adrenal tumor		
Venning et al.[11]	3	No
Gardner et al.[9,10]	3	No
D—Cushing's syndrome—adrenal hyperplasia		
Migeon et al.[31]	3	No with two Yes with one
E—Cushing's syndrome—adrenal tumor		
Migeon et al.[31]	1	No

adrenal hyperplasia and virilism treated with cortisone have been described in the literature.[5,6,10,11,14–30] In this group all but one patient (Venning[11]) showed a marked fall in urinary 17-ketosteroids as a result of therapy. After five days of oral cortisone, 200 mg. per day, Venning's patient did show a significant fall in urinary 17-ketosteroids. However, on prolonged treatment with 100 mg. a day of oral cortisone the urinary 17-ketosteroids gradually rose to pre-treatment levels. It should be noted that this patient had some of the manifestations of Cushing's syndrome, e.g., diabetic glucose tolerance curve, purple striae and osteoporosis.

A total of six cases from the literature[9–11] can be added to the herein reported five patients with adrenal virilism due to either adenoma or carcinoma. In none of these patients was there a significant fall in urinary 17-ketosteroids following cortisone administration. In one patient

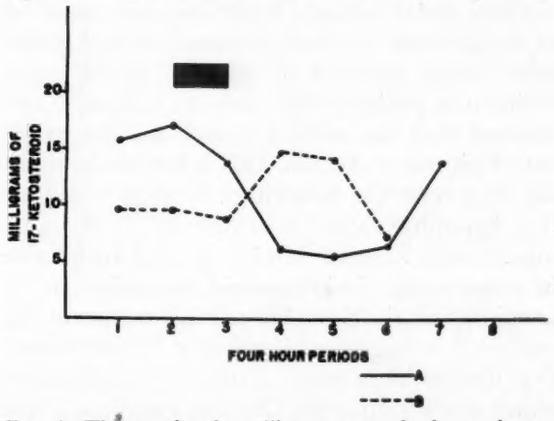

FIG. 1. The result of an "intravenous hydrocortisone test" in two patients with adrenal virilism. A, (solid line) adrenal hyperplasia; B, (broken line) adrenal carcinoma. The black bar denotes the duration of the hydrocortisone infusion.

reported by Venning[11] the decline in urinary 17-ketosteroid values was from approximately 200 mg. per day to about 100 mg. per day. The latter value is still markedly elevated and not comparable to the fall seen in patients with adrenal hyperplasia.

In regard to Cushing's syndrome, the number of cases reported in the literature (four, by Migeon and Gardner[31]) and in our series[12] is considerably smaller but it would appear that the same mechanism may be involved. It must be borne in mind that the initial pre-medication levels of the urinary 17-ketosteroids in this syndrome are not as high as those customarily found in adrenal virilism and therefore cannot show as sharp a fall. However, in five of the six cases here reported there was a significant fall; in the sixth case it is entirely possible that the patient had small multiple cortical adenomas and not adrenal hyperplasia.

Migeon and Gardner[31] have reported three patients with "idiopathic Cushing's syndrome" to whom cortisone was administered and the urinary 17-ketosteroid excretion was followed. (Table IV.) In only one patient was there a significant fall; however, the dosages of cortisone employed were smaller than those here reported. In our group of patients with adrenal tumors (Table III), and in the one case reported by Migeon and Gardner,[31] the administration of

cortisone had no effect upon the urinary excretion of 17-ketosteroids.

Since the normal adrenal gland atrophies on prolonged cortisone administration and is under pituitary control, one cannot escape the conclusion that the hyperplastic glands of adrenal virilism and Cushing's syndrome also must be under pituitary control to some degree. On the other hand, the lack of response to the same regimen in patients with adrenal tumors would indicate that the adrenal tumors are independent of pituitary control. This is further brought out by a report of Knowlton, Poole and Jailer[32] that hypophysectomy in a patient (C. R., vide supra) with adrenal carcinoma was ineffective in suppressing 17-ketosteroid excretion or in ameliorating the downhill clinical course of the patient. Furthermore, Arner et al.[33] have shown that electrocoagulation of most of the pituitary gland of a patient with Cushing's syndrome due to bilateral adrenal hyperplasia resulted in prolonged remission. A significant percentage of patients with Cushing's syndrome and adrenal hyperplasia are benefited by pituitary irradiation (literature reviewed by Plotz, Knowlton and Ragan[34]).

It is of interest to note how quickly ACTH secretion can be inhibited when hydrocortisone is administered intravenously. Within four hours after completion of an infusion the 17-ketosteroids in patient D. P. fell more than 50 per cent. In the patient with adrenal carcinoma no such fall occurred.

In view of our findings and the results reported in the literature, it would seem possible to differentiate between adrenal hyperplasia and neoplasia in the virilizing syndrome by the response of the urinary 17-ketosteroids to exogenously administered cortisone. Although the cortisone test appears to differentiate tumor from hyperplasia in most patients with Cushing's syndrome, it cannot be relied upon as fully as in the adrenogenital syndrome. The difference in the reliability of this test in Cushing's syndrome as contrasted with adrenal virilism may very well be due to the underlying steroidal abnormality inherent in these two diseased glands. In adrenal virilism the precursors of the steroids secreted are degraded to 17-ketosteroids; on the other hand, in Cushing's syndrome a greater percentage of the steroids secreted or elaborated are similar to hydrocortisone and are degraded to 17-ketosteroids to a much lesser degree. When the adrenal is inhibited by cortisone degradation of those steroids which are 17-ketosteroid precursors is also inhibited. In view of the fact that the same phenomenon occurs in both diseases as a result of cortisone administration it is safe to assume that the same underlying pituitary-adrenal axis defect is present in both conditions. The difference between the adrenogenital and Cushing's syndromes appears to lie in the inherent steroidal defect within the gland.

The differentiation between adrenal hyperplasia and neoplasia is of obvious clinical import. For this reason any test that can help make this differentiation is a valuable one.

SUMMARY*

1. The "cortisone test" has been applied to twenty-six patients with adrenal hyperplasia and ten patients with functioning adrenal tumors including instances of both adrenal virilism and Cushing's syndrome.

2. A significant fall in urinary 17-ketosteroids was obtained in all patients with adrenal virilism secondary to adrenal hyperplasia and in most instances of Cushing's syndrome due to this disorder.

3. In no instance was a fall in urinary 17-ketosteroids obtained in patients with functioning adrenal neoplasms.

4. In view of this experience and the experience reported in the literature, the "cortisone test" appears to be useful in differentiating between adrenal hyperplasia and neoplasia. Criteria for its application and interpretation are presented.

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Histopathology of Cryptorchidism*

A Study Based upon the Comparative Histology of Retained and Scrotal Testes from Birth to Maturity

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A RECENT approach to the problem of testicular maldevelopment in relation to cryptorchidism was based upon a study of a series of retained gonads.[1] Histologic evidence of testicular dysgenesis was encountered in a significant proportion. During the course of the investigation a control group of normal scrotal testes from corresponding age groups was utilized. This resulted in an accumulation of data pertaining to the microscopic anatomy of scrotal and cryptorchid testes of patients ranging in age from prematurity to the older decades. An unusual opportunity was thereby afforded to study (1) the developmental histology of the normal scrotal testes during the entire life span, and (2) the histopathology of undescended or incompletely descended testes. Since a knowledge of the former is essential for a full understanding of the latter, the value of parallel observations is self-evident. The purpose of this communication, therefore, is to compare the histologic findings in scrotal and cryptorchid testes at corresponding age levels. In this manner it is hoped to shed additional light on the histopathogenesis and management of testicular maldescent.

MATERIAL AND METHOD OF STUDY

In order to establish a base line for comparison, a group of sixty-four scrotal testes obtained from fifty-nine patients was examined. (Table I.) The age range extended from prematurity (five and one-half months) to fifty-nine years. Twenty-five were in the prepuberal period (prematurity to ten years of age), seven in the puberal era (eleven to fifteen years) and twenty-seven in the adult group. Specimens which showed gross or microscopic evidence of disease, fibrosis or atrophy had been excluded. Testicular tissue from thirty-two patients up to the age of seventeen years was obtained at autopsy. Open biopsy or ablation provided the remainder. Biopsy examinations were performed in instances of infertility, in suspected but unconfirmed hypogonadism and during the course of surgical procedures in contiguous regions. The principal reason for most of the orchidectomies was to facilitate the repair of an inguinal hernia or hydrocele. Although postmortem material imposes certain limitations on precise cytologic examination by virtue of the noxious effects of the terminal illness and tissue autolysis, it nevertheless compared favorably with the surgical preparations for the purposes of this investigation.

The undescended or incompletely descended testes of forty-two patients were studied. (Tables I and II.) The age range was from prematurity (seven months) to seventy-eight years. Nine were in the pre-adolescent period (up to ten years of age), four in the puberal range (twelve to fifteen years) and twenty-nine in adults. Cryptorchidism was right-sided in twenty-two individuals, left-sided in eleven and bilateral in nine. In the adult group, right- and left-sided involvement were present in nineteen and seven subjects, respectively, while bilaterality occurred in three. The retained glands were canalicular in twenty-four patients, intra-abdominal in thirteen and subinguinal in four. In one instance (Case 4) the right testis was intra-abdominal and the left canalicular.

The testicular specimens were obtained at necropsy in seven cases. Fresh testicular tissue was provided surgically in thirty-five patients, six by biopsy and twenty-nine by ablation. The

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TABLE I

COMPARATIVE DATA PERTAINING TO THE NORMAL SCROTAL TESTES FROM FIFTY-NINE PATIENTS AND TO A GROUP OF FORTY-TWO CRYPTORCHIDS

Age	Normal Scrotal Testes (59 Patients)*		Cryptorchidism (42 Patients)				
	Biopsy or Ablation	Autopsy	Biopsy or Ablation	Autopsy	Side		Location
					R	L	
Premature	.....	3	...	1	1	..	Inguinal
2 days	.....	1	...	1	1	..	Abdominal
1–7 mo.	.....	7	1†	3†	4	4	Inguinal (3) Abdominal (5)
4–5 yr.	1	6	...	..	..	..	
6–7 yr.	1	2	...	1†	1	1	Inguinal
10 yr.	.....	4	1	1	..	2	Inguinal
11 yr.	.....	1	...	..	..	..	.....
12 yr.	1	.....	1	..	1	..	Abdominal
13 yr.	.....	2	2‡	..	1	2	Inguinal (1) Abdominal (2)
14 yr.	.....	1	...	..	..	..	.....
15 yr.	.....	2	1	..	..	1	Inguinal
16 yr.	.....	2	...	..	..	..	.....
17 yr.	1	1 (2)	1	..	..	1	Abdominal
18 yr.	.....	.....	1	..	..	1	Inguinal
21–30 yr.	5 (7)	.....	10§	..	8	4	Inguinal (7) Abdominal (2) Subinguinal (3)
31–40 yr.	5 (6)	.....	9‡	..	6	4	Inguinal (6) Abdominal (3) Subinguinal (1)
41–50 yr.	3	.....	5	..	5	..	Inguinal (4) Abdominal (1)
51–59 yr.	10 (11)	.....	2	..	2	..	Abdominal (1) Subinguinal (1)
78 yr.	.....	.....	1	..	1	..	Inguinal
Total	27 (31)	32 (33)	35	7	Right.....22 Left.....11 Bilateral.... 9		Inguinal.....28 (24 patients) Abdominal.....16 (13 patients) Subinguinal.....5 (4 patients) Abdominal.....1 +Inguinal.....1 (1 patient)

* When both testes of a patient were examined, the total number of specimens is indicated in parentheses.
† Bilateral cryptorchidism.
‡ Bilateral cryptorchidism in one patient.
§ Bilateral cryptorchidism in two patients.

former examinations were performed in the course of investigations for suspected hypogonadism or during orchiopexy or hernioplasty. The majority of resected glands were obtained during repair of an inguinal hernia. The remainder were removed during a variety of procedures including appendectomy, suture of a perforated gastrojejunal ulcer, abdominal exploration, partial torsion of the retained testis and correction of a hydrocele. In three instances (Cases 25, 28 and 29) excision was performed because of involvement by a malignant tumor.

The majority of the patients showed no evidence of constitutional or endocrine disease. Associated congenital anomalies were encountered in four infants (Cases 2, 4, 5 and 6). Two individuals aged two months and twenty-seven years (Cases 3 and 18) were male pseudohermaphrodites. The latter was also a dwarf. Enuchoidism due to prepuberal hypogonadism was present in a twenty-three year old bilateral cryptorchid (Case 17). Shortness of stature led to a suspicion of hypogonadism in a thirteen year old boy with bilaterally undescended testes (Case 11). Involvement by malignant tumor occurred in four instances (Cases 25, 28, 29 and 33). In the last case the disease was incipient to the extent that a few small foci of early seminoma were discovered fortuitously in a small testis only after it was sectioned.

The testicular material of both groups was fixed in 10 per cent formaldehyde in most of the cases. A few biopsy specimens were fixed in Bouin's solution. All tissue was sectioned in paraffin, cut in thicknesses of 6 microns and stained with hematoxylin and eosin.

OBSERVATIONS IN THE NORMAL TESTIS

From the point of view of morphology and function the testis during the life span is a dynamic structure. The remarkable testicular alterations occurring at puberty cause the prepuberal gland to contrast sharply with that of the adult. Genuine but less obvious differences also exist between testes of individuals of the same age and in various portions of the same testes. For these reasons an accurate representation of testicular histology requires study of an adequate number of specimens distributed throughout the three principal age eras, prepuberty, puberty and adulthood. Since there is considerable physiologic variation in the age of inception and termination of adolescence of different individuals, it is evident that there can be no rigid age limits separating the three periods. However, it is rare for signs of pubescence to develop before the eleventh year or to fail to appear by the fifteenth year.[2] Accordingly, the cases in the present study are grouped as follows: fetal, prepuberty (birth to ten years of age), puberty (eleven to fifteen years) and adult (beyond fifteen years).

Fetal and Prepuberal Period. In three premature infants of five and one-half to eight and one-half months dying neonatally the testis measures 0.8 to 0.9 cm. in its longest diameter (Table III) and is composed of small, solid, non-convoluted tubules. (Fig. 1.) The average tubular diameters (derived as a mean of 5 circular cross sections) are 58, 48 and 58 microns, respectively. The seminiferous tubules contain relatively large numbers of cells of two types. These are the undifferentiated cells and the spermatogonia. The former constitute the majority and are characterized by a round or oval nucleus having fine or coarse chromatin granules and a distinct nuclear membrane. The cytoplasm is not well outlined and has a syncytium-like appearance. Scattered among the undifferentiated cells, singly or in groups, is a small number of spermatogonia. The nuclei of these cells are round, larger and have several large clumps of chromatin usually lying along the distinct nuclear membrane. The nuclei are situated centrally in a well defined mass of cytoplasm. The latter is usually clear although at times a rim of well stained denser cytoplasm encircles the nucleus. Sertoli cells are not present.

The nuclei contained within the tubules tend to be solidly packed in the smaller tubules but appear to be arranged in layers of one to three cell thickness in the larger tubules. The intertubular stroma shows considerable variation in amount and distribution at all age levels throughout the prepuberal era. It may be quite uniform with an even dispersion of discrete tubules. In other instances it is rather abundant and irregular and the tubules tend to assume a lobular arrangement. Varying patterns are often encountered in the same section.

Interstitial cells are numerous and consist primarily of fibroblasts and Leydig cells. The latter are larger, often polygonal in shape and each contains a central or eccentric nucleus. The coarse clumps of nuclear chromatin are often condensed along the distinct nuclear membrane. Certain intranuclear masses of nucleoprotein have the appearance of nucleoli.

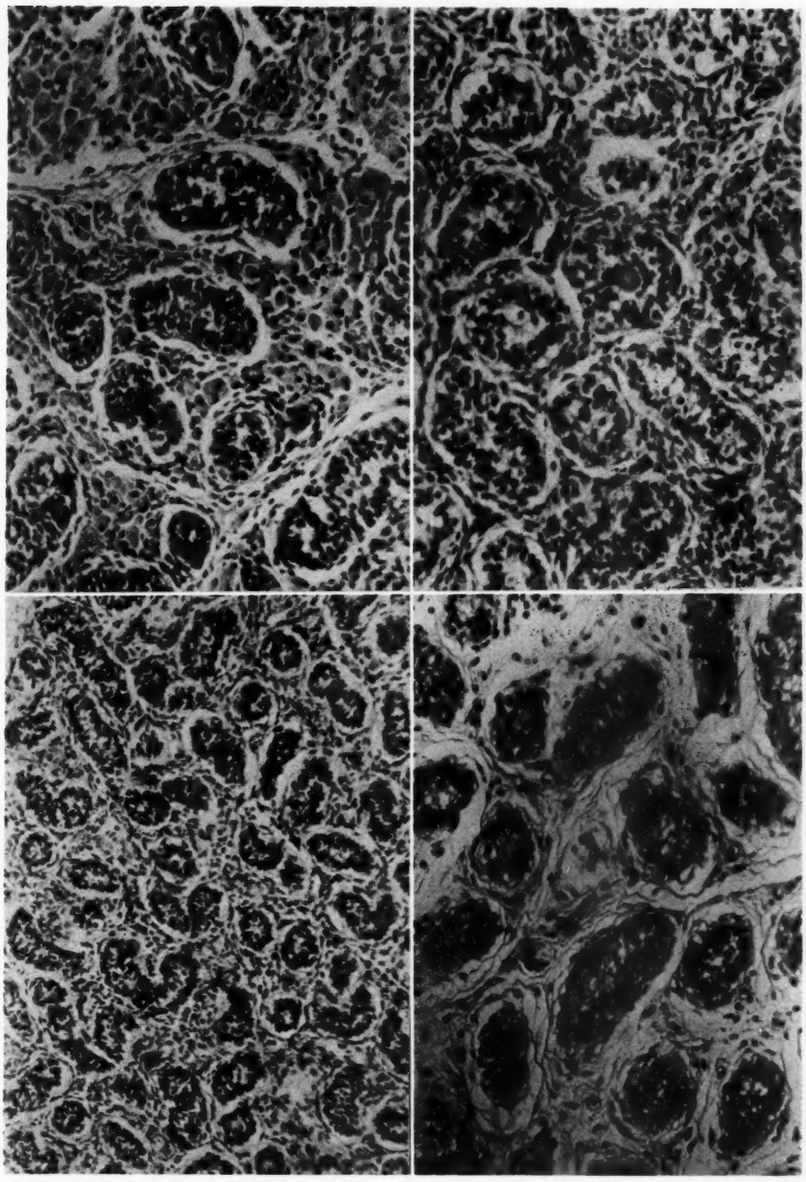


FIG. 1. Normal scrotal testis of infant born prematurely at five and one-half months. The small solid tubules are packed with undifferentiated cells and spermatogonia. The intertubular tissue contains many Leydig cells with relatively large amounts of acidophilic granular cytoplasm; hematoxylin and eosin × 225.

FIG. 2. Normal scrotal testis of infant aged six weeks. Small solid tubules containing undifferentiated cells and spermatogonia. A spheroid homogeneous, eosinophilic colloid-like body is present in a tubule in the center of the section; hematoxylin and eosin × 225.

FIG. 3. Normal scrotal testis of a five month old infant. Small solid tubules showing slight tortuosity. They are filled with undifferentiated cells and spermatogonia. It should be noted that the tubular diameters are smaller than those of the younger patients in Figures 1 and 2; hematoxylin and eosin × 225.

FIG. 4. Normal scrotal testis of four year old boy. Seminiferous tubules are small, solid and densely packed with undifferentiated cells and spermatogonia; hematoxylin and eosin × 225.

The cytoplasm is abundant, well outlined and usually granular in appearance.

Neonatally, there is little change in the histologic picture except for the gradual regression of the Leydig cells which are no longer recognizable after the first few months.

For the first decade of postnatal life very little growth or development occurs. (Figs. 2 to 6.) Of the twenty-two cases in the group of postnatal prepuberal testes the longest diameter could be ascertained in sixteen. (Table III.) In these it ranged from 0.8 to 1.8 cm. with considerable variation at any given age level. However, there was a slight over-all increment in size with advancing age. These observations are consistent with the fact that testes barely increase in weight[3] or volume[4] during this period.

The tubules show correspondingly little change in size and shape. The mean tubular diameters range from 42 to 79 microns with no correlation with age. An exception is noted in the case of a ten year old boy whose tubules averaged 100 microns in diameter. In this instance the presence of beginning Leydig and Sertoli cell differentiation and of spermatogonial mitoses heralds the onset of puberty. Since slight but definite increase in the testicular mass occurs during the prepuberal period, this must be due to an increase in the tubular length rather than width.

The tubules continue to be lined by the same two types of cells which were present antenatally, the more numerous undifferentiated cells and the less frequent spermatogonia. These are enclosed by a delicate basement membrane and in the larger tubules tend to be arranged around the periphery in layers of one to two cells.

Spermatogonia could not be identified with certainty in the case of a four year old boy from whose rather small testis a biopsy specimen was taken during an operation for hydrocele. The mean tubular diameter is 42 microns and it is quite possible that this is an instance of congenital absence of germinal cells. In another child aged seven years who succumbed four months after incurring extensive burns spermatogonia are absent, the mean tubular diameter is 49 microns and the testis measures 1.8 cm. in its longest diameter. The prolonged debilitating fatal illness probably explains the absence of germinal cells in this case.

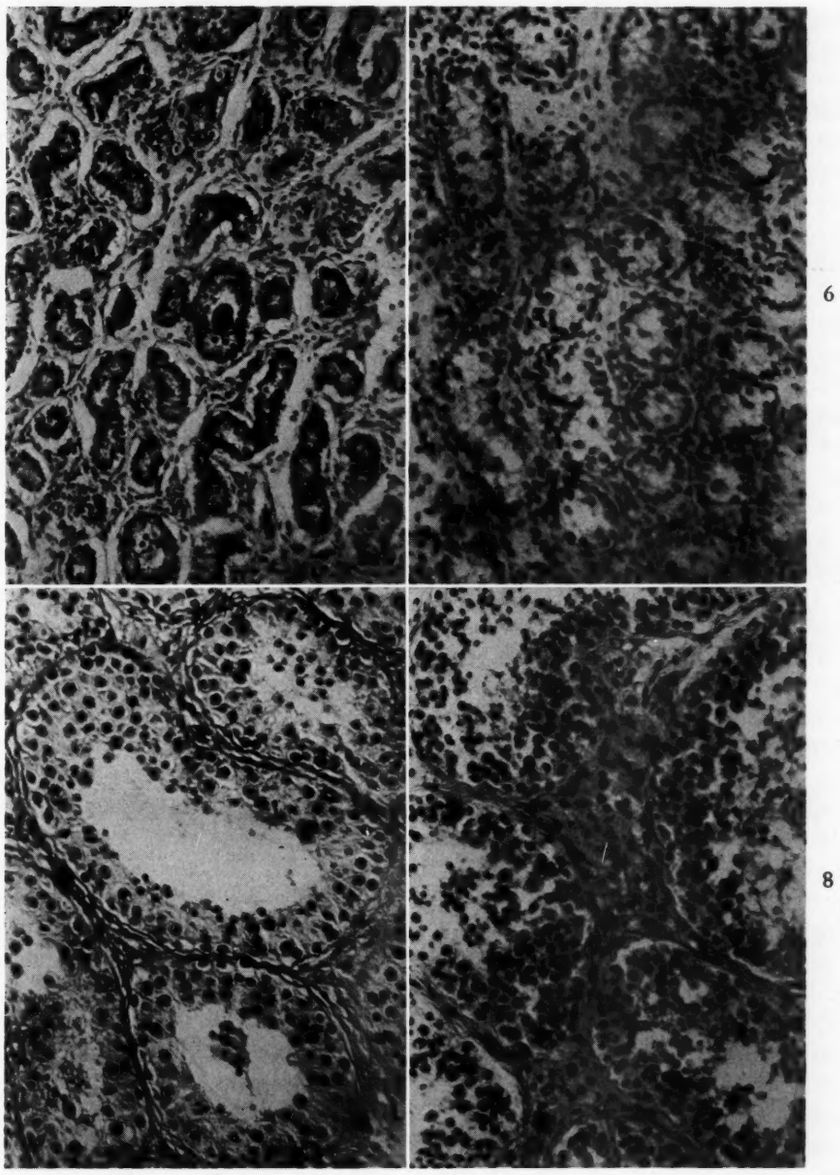
Distinct lumen formation is not encountered although small lumens have been described by other workers.[5–8] It is not unlikely that thin, faintly stained, centrally located syncytial cytoplasm may simulate an indistinct lumen, especially in the presence of cytoplasmic vacuolization or shrinkage.

Round or oval, eosinophilic, colloid-like bodies (Figs. 2 and 5) centrally situated in certain tubules were noted in four cases. These patients were aged six weeks, three months (two instances) and five years. The structures were homogeneous in most instances but occasionally showed peripheral basophilic concentric lamination or fragmentation suggestive of calcification. The tubules containing these bodies were generally larger than adjacent tubules but there appeared to be no pressure on proximate epithelial cells. The colloid-like bodies resemble corpora amylacea seen in prostatic acini. They are apparently identical with those first described in the tubules of prepuberal normal scrotal testes by Blumensaat.[9] This worker was of the opinion that they originate principally from degenerated spermatogonia although he did not exclude undifferentiated cells as a possible source.

Period of Puberty. The advent of adolescence initiates a series of graduated changes requiring a variable length of time for completion. The variations among normal prepuberal testes of the same age level are magnified during pubescence. In this group boys of the same age are apt to show marked differences in their testicular histologic patterns.

In preparation for the forthcoming events the tubular epithelial cells of the late prepuberal gland increase in size and number. The nuclei of the undifferentiated cells grow larger and more ovoid and tend to arrange themselves with their long axes at right angles to the basement membrane. Their coarse chromatin clumps become more finely dispersed creating a "ground glass" effect. The appearance of a distinct nucleolus marks the differentiation into mature Sertoli cells. This process proceeds quite uniformly throughout the tubules.

Simultaneously, mitotic figures are seen in enlarging, basally situated germinal cells denoting the inception of spermatocytogenesis. As puberty advances (Figs. 7 and 8) primary and secondary spermatocytes, spermatids and ultimately spermatozoa are formed with each new generation of cells lying more centrally than its predecessor. In contrast to the maturation of undifferentiated cells, spermatogenic activity occurs in waves in different tubules at different



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7 8

FIG. 5. Normal scrotal testis of five year old child. The tubules are lined by one or two layers of undifferentiated cells and spermatogonia. There is no definite lumen formation. A tubule in the center of the section contains a central ovoid, homogeneous, colloid-like body; hematoxylin and eosin × 225.

FIG. 6. Normal scrotal testis of a ten year old boy. The seminal tubules are small, closely set and lined by a single layer of undifferentiated cells and spermatogonia. These lie against the delicate basement membrane. Some of the larger tubules show beginning lumen formation; hematoxylin and eosin × 225.

FIG. 7. Normal scrotal testis of a twelve year old boy. The seminiferous tubules are large, convoluted and lined by several irregular layers of epithelial cells. The latter consist of differentiated Sertoli cells and several types of germinal cells. Spermatogonia lie against the basement membrane, are very numerous and show considerable mitotic activity. Primary and secondary spermatocytes and spermatids are present. Spermiogenesis has not yet occurred. Well formed lumens occasionally contain clumps of desquamated seminal epithelium. Early Leydig cell formation can be discerned in the intertubular stroma which is compressed by the enlarging tubules; hematoxylin and eosin × 225.

FIG. 8. Normal scrotal testis of a fifteen year old boy. Spermatogenesis is complete in many tubules. The seminiferous epithelium is abundant and composed of Sertoli cells and the various cells of the spermatogenic series. Spermatozoa lie near the lumen. Numerous groups of Leydig cells are present in the compact intertubular stroma; hematoxylin and eosin × 225.

times. For this reason the extent of germ cell differentiation is not uniform and varies from one tubule to another in the same testis.

With the sharp increase in cell population the tubules increase greatly in width and convolution. In the seven cases in this group the mean diameters range from 67 to 168 microns (Table III), the larger measurements corresponding to those tubules showing almost complete spermatogenesis. The contained epithelial cells become arranged in several irregular layers. Unmistakable lumens are readily recognized.

The intertubular connective tissue is compressed by the enlarging tubules. Concurrently with the maturation of Sertoli and germinal cells, the interstitial cells of Leydig make their reappearance. These are found in small groups, often surrounding a small blood vessel. They are usually polygonal in shape and have well defined cell outlines. The cytoplasm is finely granular and eosinophilic.

Postpuberal Period. With the attainment of maturity the seminiferous tubules reach a diameter of 150 to 200 microns. Full spermatogenesis is evident in most tubules. The Sertoli cells are arranged radially extending from the basement membrane toward the lumen. The cell outlines are indistinct. The cytoplasm is finely granular and eosinophilic. The cell is recognized principally by its characteristic vesicular nucleus which contains a prominent nucleolus. Mature Leydig cells are found in the loose connective tissue stroma. They are characterized by their large size, polygonal shape, distinct cell boundary and characteristic central or eccentric vesicular nucleus. The last usually contains one or two distinct nucleoli. The cytoplasm is eosinophilic and granular and is apt to be concentrated around the nucleus. Within its substance brown pigment, fine lipoid droplets and large vacuoles are frequently seen, especially toward the periphery.

OBSERVATIONS IN THE CRYPTORCHID TESTIS

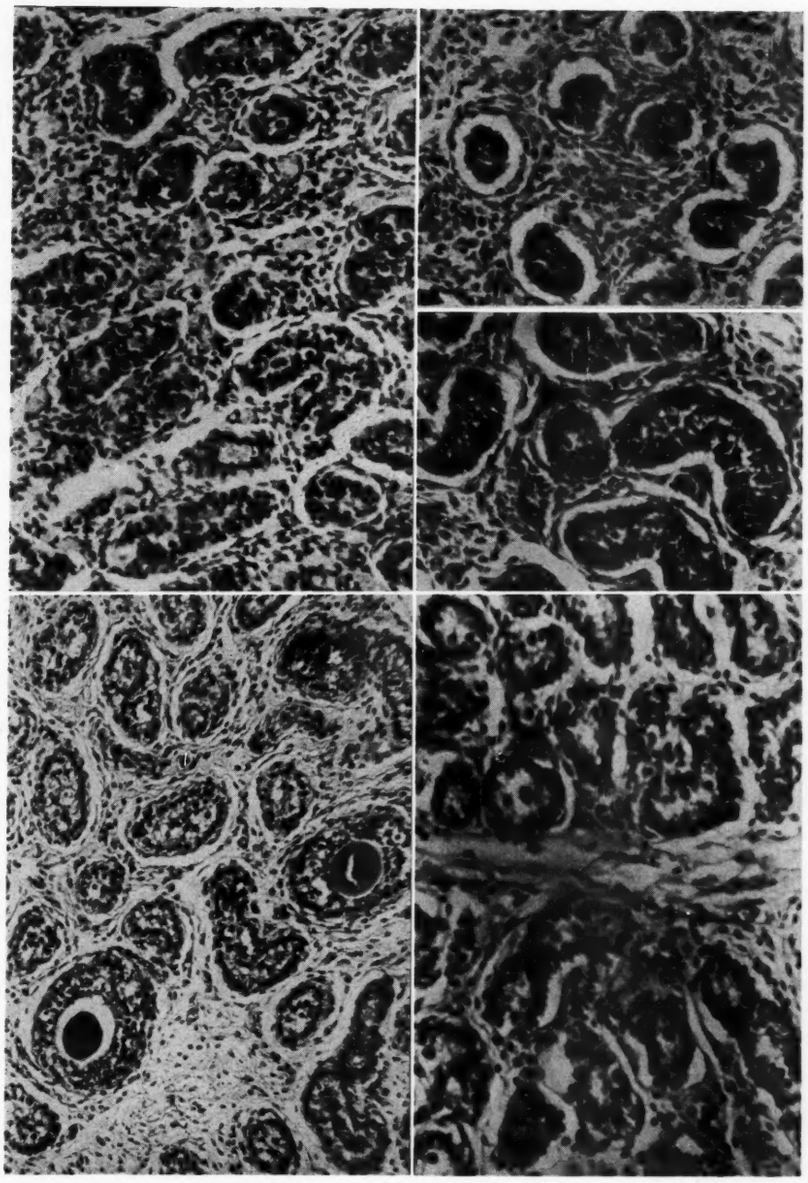
The data are subdivided into the three principal age periods with respect to puberty. This facilitates comparison with the control scrotal group and assists in the interpretation of histopathogenesis.

Fetal and Prepuberal Period. The inguinal testis of an infant born prematurely in the seventh month was studied when he succumbed neonatally. It measures 0.9 cm. in length, the average diameter of the seminiferous tubules is 57 microns (Table III) and the histologic pattern (Fig. 9) is indistinguishable from that characterizing scrotal testes during late prematurity. Of the eight remaining prepuberal cases, the gross size, mean tubular diameters and microscopic appearance (Figs. 10, 11 and 12) in six boys from two days to seven years of age are comparable to the findings in the control scrotal group for the same age range. (Tables II and III.)

Evidences of testicular inferiority were encountered in the two boys in the late prepuberal era (ten years of age). The inguinal testis in Case 8 is approximately two-thirds the size of its scrotal mate and their average tubular diameters are 55 and 100 microns, respectively. (Fig. 13.) The tubular cell population of the retained testis is sparser and arranged in fewer layers around the periphery. Lumens, typical undifferentiated cells and spermatogonia are present in both testes. However, in the descended gland the nuclei of the undifferentiated cells tend to be larger and some show faint nucleolus formation signifying beginning Sertoli cell maturation. Spermatogonia are more numerous and show evidence of mitotic activity. The intertubular connective tissue stroma appears to be compressed by the enlarging tubules. On the other hand it is abundant and loose and shows a tendency to peritubular condensation in the contralateral retained gonad. There is no thickening of the basement membrane or tunica propria of any of its tubules. An occasional interstitial cell of Leydig is recognized in the scrotal but not in the cryptorchid testicle.

In the other ten year old boy (Case 9) the tubules of the canalicular testis are completely lacking in spermatogonia. The tubules show no fibrosis and are tightly packed with numerous undifferentiated cells, some of which are enlarging. However, none show plasmosomes indicative of Sertoli cell differentiation. Occasional nests of typical Leydig cells containing lipoid droplets and vacuoles are present in an intertubular connective tissue stroma which is abundant and free from fibrosis.

Period of Puberty. Efforts to compare the observations in four cryptorchid and seven scrotal testes in this age period are hampered by the relatively small number of cases. No definite statement can be made concerning the comparative sizes of testes. The mean tubular diameters vary considerably in each group but tend to be larger in the scrotal glands.



9 10

11 12

FIG. 9. Inguinal testis of infant born prematurely at seven months (Case 1). The histologic pattern is identical with that of scrotal testes at the same age. The small solid tubules are packed with undifferentiated cells and spermatogonia. The intertubular connective tissue contains fibroblasts and Leydig cells; hematoxylin and eosin × 225.

FIG. 10. *Upper:* Intra-abdominal testis of two day old infant (Case 2). *Lower:* Contralateral scrotal testis in same patient. The histologic pattern is essentially the same. The differences in tubular diameter and in stromal distribution, cellularity and density are within the normal range for the prepuberal period. The intratubular epithelial cells are numerous and consist of undifferentiated cells and spermatogonia; hematoxylin and eosin × 225.

FIG. 11. Intra-abdominal testis of seven month old infant (Case 6). The histologic picture is identical with that of normal prepuberal scrotal testes. Spheroid or ovoid colloid-like bodies are centrally situated in two tubules. One shows basophilic lamination and fragmentation suggestive of calcification; hematoxylin and eosin × 225.

FIG. 12. Inguinal testis of seven year old boy (Case 7). The histology does not differ from that of normal scrotal testes (Figs. 4 and 5) of the same age period; hematoxylin and eosin × 225.

TABLE II

CORRELATION BETWEEN AGE, LOCATION AND HISTOPATHOLOGIC FINDINGS IN FORTY-TWO CRYPTORCHID TESTES

Case No.	Age	Location	Fibrosis*		Germinal Cells**	Remarks
			Tubular	Intertubular		
1	Premature (7 mo.)	Inguinal	0	0	3	.....
2	2 days	Abdominal	0	0	3	Associated congenital anomalies
3	2 mo.	Inguinal	0	0	3	Pseudohermaphrodite
4	4 mo.	Abdominal	0	0	3	Associated congenital anomalies
5	6 mo.	Abdominal	0	0	3	Associated congenital anomalies
6	7 mo.	Abdominal	0	0	3	Associated congenital anomaly
7	7 yr.	Inguinal	0	0	3	.....
8	10 yr.	Inguinal (mid)	0	0§	2	⅔ size of contralateral scrotal testis
9	10 yr.	Inguinal	0	0§	0	Germinal aplasia
10	12 yr.	Abdominal	0	+§	2	Immature tubules
11	13 yr.	Abdominal	0	0	2	Immature tubules
12	13 yr.	Inguinal (mid)	0	0	2	.....
13	15 yr.	Inguinal (low)	+	0	2, 4	.....
14	17 yr.	Abdominal	+	+	1	Immature tubules
15	18 yr.	Inguinal	+	+	0	Immature tubules
16	21 yr.	Inguinal	+	++	1	.....
17	23 yr.	Inguinal (high)	0	0	0	Prepuberal hypogonadism; absence of germinal, Sertoli and Leydig cells
18	27 yr.	Subinguinal	0	0	2, 5	Pseudohermaphrodite; dwarf
19	28 yr.	Inguinal	+†	0	1	Immature tubules
20	28 yr.	Inguinal	++	++	1	Immature tubules
21	29 yr.	Inguinal	+++‡	++	0	.....
22	30 yr.	Inguinal	++	+	0	Immature tubules
23	30 yr.	Abdominal	++†	+++	0	Immature tubules
24	30 yr.	Subinguinal	++	+++	1	.....
25	30 yr.	Abdominal	+++‡	+++	1	Seminoma; immature tubules
26	31 yr.	Inguinal (high)	+++	+	1	Immature tubules
27	33 yr.	Abdominal	++†	+	?	.....
28	34 yr.	Inguinal	+++‡	++	0	Seminoma
29	35 yr.	Subinguinal	—	—	—	Teratoma (entire gland destroyed)
30	36 yr.	Inguinal	+ to +++†	+++	1	Immature tubules
31	36 yr.	Inguinal	+++‡	+	0	.....
32	38 yr.	Inguinal (low)	+++‡	+	1	Immature tubules
33	39 yr.	Abdominal	+++‡	+§	1	Seminoma; immature tubules
34	39 yr.	Abdominal	+++†	+++§	0	.....
35	41 yr.	Abdominal	++	++§	?	.....
36	42 yr.	Inguinal (low)	+†	+	2, 6	.....
37	42 yr.	Inguinal	+	+§	?	.....
38	48 yr.	Inguinal	++†	+	1	Immature tubules
39	50 yr.	Inguinal (high)	+ to ++‡	+	0	.....
40	52 yr.	Abdominal	+++‡	+++§	0	Immature tubules
41	52 yr.	Subinguinal	++	+	0	.....
42	78 yr.	Inguinal (low)	+ to +++†	0	0	.....

* Key to description of fibrosis:
0 —Absent
\+ —Slight
++ —Moderate
+++—Advanced
§ Intertubular stroma abundant.
† A few tubules are totally obliterated by fibrosis.
‡ Many tubules are totally obliterated by fibrosis.

** Key to description of germinal cells:
?—Presence of spermatogonia uncertain (degeneration or autolysis)
0—Absent
1—Spermatogonia very infrequent
2—Spermatogonia moderately decreased in number
3—Spermatogonia in normal number
4—Spermatogenic arrest at primary spermatocyte stage
5—Spermatogenic arrest at spermatid stage
6—Spermatozoa present

Significant differences are apparent in the cellular composition of the seminiferous tubules. (Table II.) The number of spermatogonia is diminished in all four cryptorchid glands. There is no evidence of mitotic activity in the germinal cells in Cases 10, 11 and 12. Case 13, a fifteen year old boy, shows spermatocytogenesis but on a reduced scale. In general, the tubular cell population in all four cases appears sparser than normal. Slight sclerotic thickening of the basement membranes is noted in one patient (Case 13). Very slight fibrosis of an abundant intertubular connective tissue stroma is present in Case 10. (Fig. 14.)

Sertoli cell differentiation is evident in all cases despite retarded germ cell maturation. Recognizable interstitial cells of Leydig are not found in Case 12 but are present in the others.

Postpuberal Period. The adult cryptorchid testis is abnormal in each of the twenty-nine cases. Total teratomatous destruction occurred in Case 29 so that no uninvolved portion was available for study. Degeneration of the seminal epithelium, tubular atrophy and fibrosis of the tubular and intertubular structures constitute the characteristic histologic pattern. However, there are striking variations in the extent and intensity of the morbid process from patient to patient. Furthermore, notable differences are frequently evident in different areas of the same section.

Inhibition of spermatogonial activity, first noted in the puberal period, is now more pronounced. (Table II.) Spermiogenesis is noted only once (Case 36). Spermatogenic arrest at the spermatid stage is present in Case 18. Spermatogonia are nowhere present in normal numbers. They are absent in twelve instances, questionably present in three and occur in reduced numbers in the remainder. Degeneration and desquamation of the germinal cells is frequent.

Evidence of germinal cell deterioration bears no impressive relationship to the age of the patient. Although there is a tendency for it to be more marked at the older age levels, there are frequent exceptions, as in the forty-two year old patient with spermatozoa. The occurrence of spermiogenesis in a low canalicular testicle and spermatids in a subinguinal gland suggests that location may be related to the degree of germ cell impairment. However, this does not appear to be an important factor in distinguishing between testes situated intra-abdominally or in the upper half of the inguinal canal.

By comparison, Sertoli cells display a remarkable ruggedness in resisting the deleterious effects of cryptorchidism. Although in reduced numbers, frequently showing evidence of cytoplasmic and nuclear degeneration, they persist for some time after the germinal cells have disappeared. Most often a single discontinuous layer of cells lines the shrinking tubules. Ultimately, these vanish as the latter become completely obliterated.

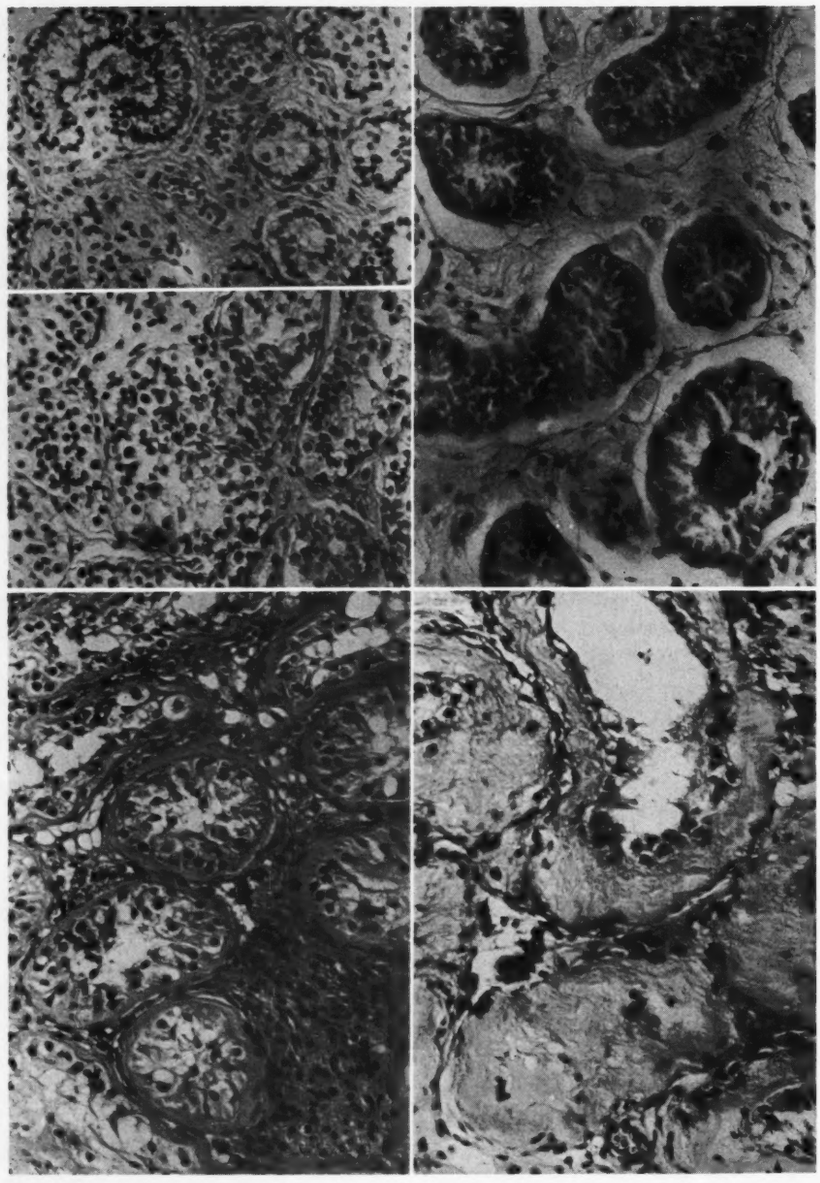
TABLE III

COMPARISON OF SIZE OF TESTES AND TUBULAR DIAMETERS IN PRE-ADULT SCROTAL AND CRYPTORCHID GLANDS

	Normal Scrotal Testes		Cryptorchid Testes	
	Greatest Diameter (cm.)	Mean Tubular Diameter (microns)	Greatest Diameter (cm.)	Mean Tubular Diameter (microns)
Fetal.....				
5½ mo....	0.8	58	...	...
7 mo.....	...	..	0.9	57
8½ mo....	0.8	48	...	...
8½ mo....	0.9	58	...	...
Prepuberal				
2 days.....	...	56	0.9	65
5 wk.....	0.8	60	...	...
6 wk.....	1.1	59	...	...
6 wk.....	0.8	71	...	...
2 mo.....	...	..	...	40
3 mo.....	1.1, 1.0	74, 79	...	...
4 mo.....	...	..	1.0	50
5 mo.....	1.3	50	...	...
6 mo.....	1.1	61	1.1	60
7 mo.....	...	..	1.3	75
4–5 yr.....	1.4, 1.7, 1.0 1.5, 1.1	60, 59, 49 50, 50 42, 56	...	...
6–7 yr.....	1.4, 1.8, 1.6	54, 49, 48	1.0	65
10 yr.....	1.1	100	0.7	55
		65, 72, 65	1.3	65
Puberal				
11 yr.....	1.3	67	...	...
12 yr.....	...	168	2.0	90
13 yr.....	1.9, 2.9	94, 156	1.5	82 136
14 yr.....	...	99	...	...
15 yr.....	2.0	125 161	...	136

Concomitantly, the seminiferous tubules undergo progressive fibrosis and atrophy. The earliest changes are seen as a sclerotic thickening of the basement membrane. (Fig. 15.) As the process advances, the tunica propria participates and a widened hyalinized tubular wall results. Extension of fibrosis occurs centrally with encroachment on the epithelial contents and lumen. With eventual fibrous replacement the entire tubule is transformed into a small dense, hyalinized, relatively acellular structure. (Fig. 16.)

Although tubular fibrosis appears to be



13 14

15 16

FIG. 13. *Upper:* Inguinal testis of a ten year old boy (Case 8). *Lower:* Contralateral scrotal testis in the same patient. The cryptorchid gland shows smaller tubules containing fewer numbers of epithelial cells. Its undifferentiated cells and spermatogonia show no evidence of maturation and the intertubular stroma contains no Leydig cells. On the other hand, the scrotal gland shows beginning Sertoli and germ-cell maturation, early Leydig cell differentiation and compression of the intertubular stroma by the enlarging tubules; hematoxylin and eosin × 225.

FIG. 14. Intra-abdominal testis of twelve year old boy (Case 10). The majority of tubules are of the prepuberal type containing undifferentiated cells and spermatogonia. A central round homogeneous colloid-like body is present in one tubule. The intertubular stroma is fairly abundant and shows slight fibrosis. There are no Leydig cells. Elsewhere (not shown) small foci of maturing tubules containing differentiated Sertoli cells are present; hematoxylin and eosin × 225.

FIG. 15. Inguinal testis of thirty year old man (Case 22). The seminiferous tubules are shrunken, have a sparse cellular content and are lined by one or two layers of Sertoli cells. Germinal cells have disappeared. Slight to moderate fibrotic thickening of the basement membranes is present. The intertubular connective tissue is slightly fibrotic. A large accumulation of Leydig cells is seen in one corner of the section; hematoxylin and eosin × 225.

FIG. 16. Inguinal testis of a thirty-eight year old man (Case 32). Tubular fibrosis is advanced. The basement membrane and tunica propria of most tubules are markedly thickened by the dense hyalinized fibrotic process. Residual seminal epithelium consists of a single, discontinuous layer of Sertoli cells, many of which show nuclear and cytoplasmic degeneration. Germinal cells have disappeared. Many tubules are completely obliterated and transformed into small, hyalinized, relatively acellular fibrotic structures; hematoxylin and eosin × 225.

progressive, the tubules are not involved uniformly. Varying degrees are evident in different fields of the same section. Total fibrotic obliteration of a few tubules is seen in some cases showing only slight fibrosis of the other tubules. On the other hand, an advanced degree of tubular fibrosis may or may not be accompanied by complete obliteration of some tubules. In general, however, the number of fully obliterated tubules is directly proportional to the over-all intensity of tubular fibrosis.

The extent and intensity of tubular fibrosis appear to increase as the fourth decade is approached. Earlier it is absent in two patients and slight in the others. The youngest individual showing advanced tubular fibrosis was twenty-nine years of age. Thereafter, no definite correlation is apparent between age and amount of tubular fibrosis. Similarly, the situation of the cryptorchid testis appears to exert no definite bearing on the amount of tubular fibrosis.

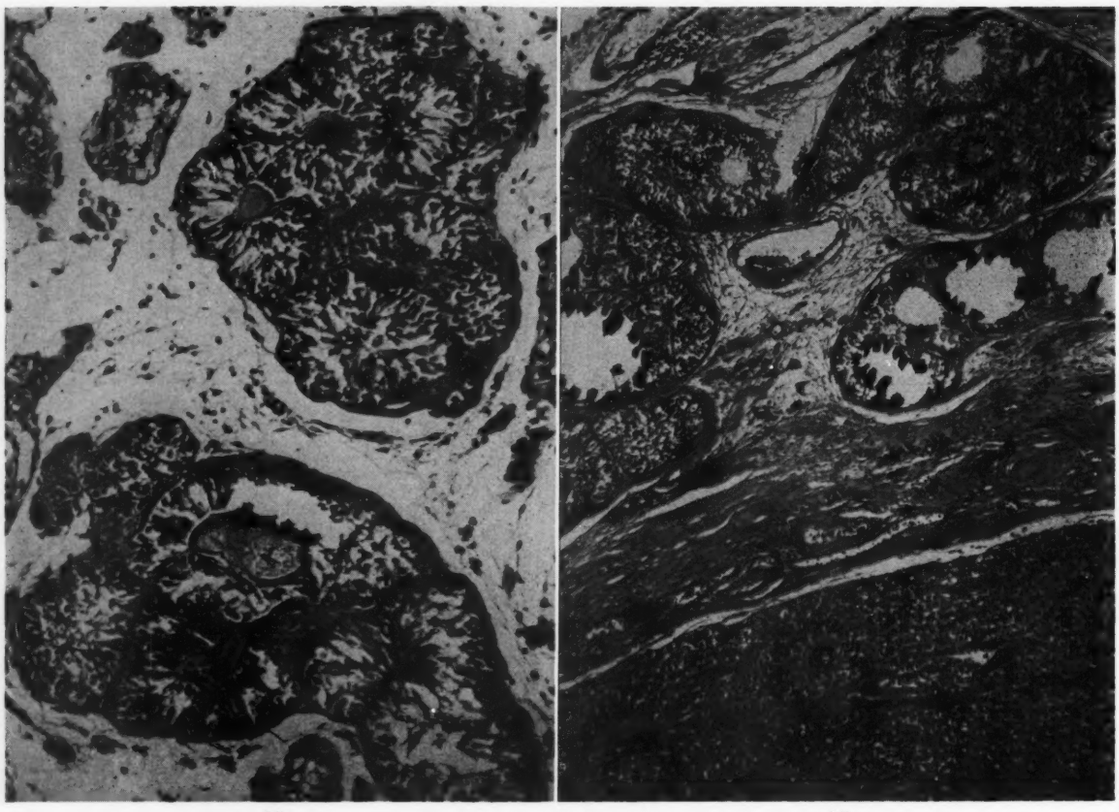
The intertubular tissue displays considerable variation. It is quite abundant in some instances. In the majority of cases it shows varying amounts of fibrosis. Peritubular fibrosis is common. This often merges with irregular, dense, hyalinized, relatively acellular fibrotic areas. Three patients in the third and one in the eighth decade show no fibrosis. Although the younger adults tend to have less intertubular fibrosis there is no apparent correlation with age beyond the third decade. A relationship between the amount of fibrosis and the location of the retained gland could not be established.

Interstitial cells of Leydig show no important qualitative alterations. However, there is considerable variation in their numbers. They are sparse in some instances, normal in others but most often show an apparent numerical increase. Conspicuous focal aggregations are frequently seen. Occasionally these may be quite large and circumscribed and may simulate a microscopic adenoma. This observation favors the impression that an apparent increase in number may be due, at times, to actual hyperplasia as well as to condensation.

Sclerotic thickening of arteriolar walls is present in approximately one-third of the specimens. The earliest appearance of this finding is at the age of twenty-nine years which coincides with the earliest appearance of advanced tubular fibrosis. Thereafter, arteriolosclerosis, when present, is associated with moderate or advanced tubular fibrosis. It is not correlated with advancing age, location of the cryptorchid testis or the extent of intertubular fibrosis.

FIG. 17. Inguinal testis of eighteen year old man (Case 15). Two types of tubules are present. The smaller solid tubules are of the prepuberal type containing large numbers of undifferentiated cells but no spermatogonia. A tubule near the center of the section shows a central round colloid-like body. These tubules are relatively free of fibrosis, and the contained epithelial cells are well preserved. Immediately adjacent are several larger tubules which have previously matured but now show the deleterious effects secondary to cryptorchidism. They appear shrunken and contain a relatively sparse number of cells. The latter consist entirely of Sertoli cells, some of which are degenerating. These tubules show early fibrotic thickening of the basement membranes; hematoxylin and eosin × 225.

Evidence of Testicular Dysgenesis. Histologic criteria for the recognition of congenital testicular defects have been described previously.[1,10–12] They are based upon two principal considerations derived from a study of normal testes in patients ranging in age from birth to maturity. First, the verified absence of cytologic components such as spermatogonia, Sertoli or Leydig cells unattributable to age, cryptorchidism or postnatal factors. Second, the persistence beyond the prepuberal era of immature seminiferous tubules. These resemble the prepuberal type in



18 19

FIG. 18. Inguinal testis of twenty-eight year old man (Case 19), showing compact, sharply circumscribed groups of tubules of the prepuberal type. These have a nodular or lobulated appearance. The individual tubules are solid and densely packed with undifferentiated cells. Spermatogonia are absent. Several tubules contain ovoid colloid-like bodies. The tubules show little or no fibrosis and the undifferentiated cells are well preserved. Scattered nearby are a few atrophic and fibrotic seminiferous tubules containing a sparse number of Sertoli cells; hematoxylin and eosin × 225.

FIG. 19. Intra-abdominal testis of a thirty year old man (Case 25). A large part of this gland was involved by seminoma shown at lower edge of the section. The uninvolved portion near the surface contains several compact, sharply circumscribed groups of tubules of the prepuberal type. These are densely populated by fairly well preserved undifferentiated cells. Distinct lumens are absent but many tubules contain fragmented remnants of calcified material. An occasional tubule contains a central, smaller, homogeneous colloid-like body. Slight fibrotic thickening of some of the basement membranes is present. The adjacent connective tissue stroma is markedly fibrotic. Scattered within it are several atrophic and fibrotic tubules containing a few degenerating Sertoli cells; hematoxylin and eosin × 100.

that they contain undifferentiated rather than Sertoli cells.

Evidence of defective gonadogenesis in the present series of cryptorchid testes is presented in detail elsewhere.[1] Three varieties are recognizable histologically. In ascending order of frequency these are:

1. Total absence of germinal, Sertoli and Leydig cells in a twenty-three year old eunuchoidal man with bilateral cryptorchidism (Case 17).

2. Complete absence of spermatogonia in a ten year old boy (Case 9).

3. Various degrees of tubular immaturity occurring after the prepuberal age. Seminal tubules of a prepuberal type (Fig. 17) are identified in the retained testes in sixteen of thirty-two subjects beyond the age of ten years. These tubules are small and often comparable in size to the mature tubules which show the atrophy and fibrosis characteristic of cryptorchidism. They are readily distinguishable from the latter by the fact that they show little, if any fibrosis. In addition, the contained undifferentiated cells are relatively well preserved. Central spheroid colloid-like masses (Figs. 11, 14, 17 to 19) enhance the resemblance to the prepuberal type of seminiferous tubules. The immature tubules are

usually scattered singly or in small groups. However, focal grouping presents a distinctive variation in three patients (Cases 19, 25 and 30) in that the tubules are closely set in compact, sharply circumscribed units with a nodular or lobulated appearance. (Figs. 18 and 19.)

COMMENT

The histopathologic alterations encountered in the adult cryptorchid testis have been the subject of numerous reports in the literature. In general, the results of the present study agree with the carefully documented microscopic descriptions by Wangensteen[13] in seventeen cases, Cooper[14] in twelve, Pace and Cabot[15] in twenty-four, Rea[16] in forty-six and Nelson[17] in twenty-two. However, the great majority of these observations were made in adult or adolescent individuals. Data derived from studies of prepuberal cryptorchid testes are meager and controversial. Moreover, little or no attention has been devoted to defective gonadogenesis as an etiologic factor in cryptorchidism. Finally, the problem of the relationship between tumor and retention of the testicle is in need of further study.

Many workers[13,16,18–22] state that retained and scrotal prepuberal testes are anatomically identical. Others[14,17,23–25] note histologic evidence of inferiority in certain undescended glands before puberty. The observations in the present study point to an explanation of these divergent points of view. In part, they confirm the opinion of Uffreduzzi,[25] Cooper[14] and Nelson[17] that differences between scrotal and retained prepuberal testes are discernible. These are subtle and would readily escape detection unless specifically searched for. For example, the canalicular testis of a ten year old patient (Case 8) appeared to be normal in all respects until it was compared with the contralateral scrotal gland. It was then apparent that the retained testis was smaller with a corresponding diminution in the size and cell population of its tubules. In addition, the seminal epithelial cells exhibited a comparative retardation of maturation. The complete absence of spermatogonia in another ten year old boy (Case 9) strongly suggests a congenital defect (germinal aplasia) since their disappearance as a result of malposition is not definitely known to occur before late adolescence. It is not unreasonable to expect that when larger series of prepuberal testes are critically examined germinal aplasia will be recognized earlier in the prepuberal period.

The existence of considerable variation in the size of the tubules and amount of intertubular stroma among boys of the same age during the prepuberal era accounts for additional discrepancies in the literature. Observations in the control group of scrotal testes confirm Wangensteen's[13] opinion that the "primary atrophy" described by Félizet and Branca[23,24] in certain prepuberal cryptorchid testes is a normal finding before puberty. It is obvious, therefore, that tubular size and stromal quantity by themselves are not reliable indices of morbidity in prepuberal glands.

The occurrence of histologic evidence of dysgenesis in approximately one-half of the retained testes from patients ten years of age and older probably has an important bearing on the etiology, histopathogenesis and treatment of cryptorchidism. The frequency with which testicular dysgenesis was encountered supports the concept that inherent imperfection of the gland itself may be partially or wholly responsible for maldescent. Since congenital anomalies are often multiple they may involve anatomical structures concerned with the pathway of testicular descent and the endocrine system as well as the gonad itself. Under these circumstances the role of testicular imperfection in the etiology of cryptorchidism may be contributory, subsidiary or incidental.

The presence of immature seminiferous tubules in an adult's cryptorchid testis has been recorded previously. "Embryonal" tubules were described in one case by Uffreduzzi[25] in 1913 and Sniffen[6] has recently commented on a similar finding. Tubular immaturity is significant evidence of testicular dysgenesis and modifies the histopathologic pattern of the adult cryptorchid testis. Their relative immunity to fibrosis and epithelial degeneration is probably responsible for the fact that the presence and significance of immature tubules in the retained testis have not been generally recognized.

The distinctive nodular appearance of sharply circumscribed units of compact immature tubules noted in Cases 19, 25 and 30 merits special comment. As described elsewhere[1] they bear a striking superficial resemblance to the *testicular adenomas* described and illustrated by Pick[26] and Lecène and Chevassu[27] in undescended testes. However, detailed histologic analysis indicates that these two types of nodular formations in cryptorchid testes are unrelated. The ostensible similarity of the congenital

"nodular" lesions to neoplasia is stressed in view of a report in the literature.[15] In this instance it appears that characteristic microscopic aggregates of immature tubules were interpreted as early adenocarcinoma in retained testes. Apparently identical aggregations in a cryptorchid testis are interpreted by Scully and Parham[28] as testicular adenomas, with the reservation that they are probably not true tumors.

A primary malignant testicular tumor was encountered in four of forty-two cryptorchid testes, of which twenty-seven were from adults. Despite the fact that the material is of surgical and postmortem origin and the statistics are by no means representative of the general incidence of tumor in retained testicles, the tumor frequency in the present series is, nevertheless, quite impressive. This is particularly so in view of the fact that the patients were observed in a general hospital. Furthermore, the tumor was a fortuitous discovery in two of the four instances, being unsuspected prior to operation. In Case 25 an asymptomatic intra-abdominal seminoma was revealed during the course of an appendectomy. In Case 33 a small atrophic intra-abdominal testis was removed during the repair of a strangulated inguinal hernia. It was not until this gland was sectioned that very small foci of seminoma were discovered. The patient in Case 28 was operated upon because of a painful slightly enlarged inguinal testis and the tumor was recognized at that time.[29]

Although this series is admittedly small and unaccompanied by control statistics pertaining to the distribution of tumors among scrotal and cryptorchid glands, the observations appear to confirm the majority opinion in the literature. In a survey of more than 7,000 malignant testicular tumors, Gilbert and Hamilton[30] found 11 per cent in men with cryptorchid testes. Campbell[31] and Twombly[32] reported similar figures. Since the incidence of retained testes in the adult population has been estimated to be in the neighborhood of 0.2 per cent,[30] it appears that malignancy is encountered approximately fifty times as frequently in the cryptorchid as in the scrotal testis.

That malposition by itself is responsible for the increased occurrence of neoplasm is rather doubtful. The development of a tumor in a testicle some time after orchiopexy[33] or late spontaneous descent[13] suggests other factors. The same applies to recorded instances[30] of tumors of the scrotal testis in men whose other testicle was undescended. The possibility of congenital or constitutional inferiority of the testis appears to be a more likely explanation. This is supported by the high incidence of testicular dysgenesis in the present study of cryptorchid glands. In Case 33 evidence of defective gonadogenesis was also found within the substance of a very small focus of seminoma. The occasional occurrence of neoplasm in the descended testis of an unilateral cryptorchid[13,30] and especially in the undescended testes of male pseudohermaphrodites[30,34] strengthens the concept of constitutional inferiority. An additional link in the chain of evidence is the fact that the brother of Case 24 had a seminomatous cryptorchid testis[35] suggesting a familial constitutional inferiority in this instance. This is not surprising in view of the existence of familial cryptorchidism.[36–38]

It is highly probable that the foregoing considerations have an important bearing on certain aspects of the therapy of cryptorchidism. Defective gonadogenesis, expressed as testicular incompetence, may be partially or wholly responsible for the failure of hormonal treatment. It may also contribute to the notoriously poor results with respect to improving the fertility potential after orchiopexy. These have been summarized by Nelson.[17] Since evidence of retarded maturation in the present study was encountered at the tenth year of age, it seems advisable to consider surgical therapy before this time if a preliminary trial with hormonal treatment fails. On the basis of personal observations of decreased numbers of spermatogonia in the prepuberal cryptorchid testis Nelson[17] recommends that therapy not be delayed beyond the age of six or seven years.

Data from the present series of cases indicate that spermatogenic activity is generally markedly impaired even in the young adult cryptorchid. However, significant tubular fibrosis was not observed until the thirtieth year was approached. Beyond this age the histopathologic process was generally so advanced as to suggest irreversibility. It would seem, therefore, that orchiopexy for the purpose of improving fertility is probably futile if performed later than the third decade. Apparently this might not apply to testicles situated low in the inguinal canal or just outside of the external ring. The two glands showing the least amount of tubular changes were of practically normal size and were located in these regions. Similar observations pertaining

to testicles situated near the external ring were recorded by Rea.[16]

From the point of view of possible subsequent malignancy, the management of the cryptorchid patient beyond the age of thirty years has been the subject of considerable concern and controversy. Conceding the incidence of testicular cancer to be very low (0.0013 per cent[30] for living males), nevertheless it appears to occur about fifty times as often in cryptorchids as in men with scrotal testes. Furthermore, the likelihood of neoplastic involvement of the other testis in men who have had unilateral involvement is much greater in retained than in scrotal glands.[39] Despite these observations, many workers are of the opinion that the incidence is still too low to justify prophylactic orchidectomy.[13,40] However, the occurrence of four cases in the present series is sufficiently impressive to warrant a reconsideration of this viewpoint. This applies particularly to patients with unilateral cryptorchidism where there is no risk of inducing a castration syndrome. Moreover, since an intra-abdominal testis is several times as prone as an inguinal gland to malignant transformation[31,39] an abdominally retained testicle should be viewed with considerable concern.

SUMMARY AND CONCLUSIONS

1. The undescended or incompletely descended testes of forty-two patients were compared histologically with a control group of sixty-four normal scrotal testes obtained from fifty-nine patients. Since the age range in both groups extended from prematurity to adulthood, an opportunity was afforded to study the developmental histology of the normal testis as well as the histopathology of the retained gland.

2. The growth and development of the normal testicle is barely perceptible from birth to puberty. The seminiferous tubules are solid and contain undifferentiated cells and spermatogonia. There is considerable normal variation in tubular diameter, epithelial cell density and stromal mass and cellularity among boys of the same age level. The advent of puberty is characterized by a rather rapid increase in tubular diameter, the initiation of spermatogenesis, full maturation of Sertoli cells from undifferentiated cells and the appearance of Leydig cells in the intertubular stroma which becomes compressed by the enlarging tubules.

3. There were discernible differences between retained and scrotal prepuberal testes. These were subtle and noted toward the end of the prepuberal period. They consisted of a decrease in tubular size and cell population in the cryptorchid gland. In addition, maturation of the seminal epithelium was retarded. Total absence of spermatogonia was observed in one patient suggesting a primary congenital defect (germinal aplasia).

4. Distinctive histopathologic alterations were present in cryptorchid testes during the puberal age. They consisted primarily of a moderate retardation of the spermatogenic process. A tendency to fibrosis of the basement membrane and peritubular connective tissue was present. Differentiation of Sertoli and Leydig cells apparently proceeded normally.

5. Progressive changes were noted early in adult life. These were characterized by varying degrees of degeneration of the seminal epithelium, tubular atrophy and fibrosis of the tubular and intertubular structures. Germinal cell deterioration tended to be more marked at older age levels with frequent exceptions. It was least apparent in two testicles situated near the external inguinal ring but otherwise showed no relation to testicular location. Tubular fibrosis increased rather sharply with the approach of the fourth decade. Thereafter, there was no definite correlation with age.

6. Histologic evidence of defective gonadogenesis was found in approximately one-half of the retained testes while it was virtually absent from the control group. (In the single instance of a four year old boy with a rather small scrotal testis, there appeared to be evidence of germinal aplasia, presumably a congenital defect.) It is believed that testicular dysgenesis may play an important role in the etiology of certain cases of cryptorchidism and may have an equally important bearing upon therapy. It may account for the failure of hormonal treatment to induce descent and the failure of orchiopexy to improve the fertility potential. The frequent presence of congenital testicular defects in cryptorchid testicles may be related in some way to the increased incidence of malignancy in undescended as compared with scrotal testes.

7. The occurrence of four instances of testicular cancer in the present group of forty-two cryptorchid patients is noteworthy although by no means representative of the true incidence rate for cryptorchids in general. Nevertheless, it suggests that prophylactic orchidectomy be

given serious consideration, particularly in the patient with unilateral intra-abdominal cryptorchidism.

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Genetic Aspects of Adenomatosis of Endocrine Glands*

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THE simultaneous occurrence of adenomas of the anterior pituitary, the parathyroids and the islet cells of the pancreas in one individual has been known as a pathologic rarity for many years[1–4] but has lately also claimed the interest of the clinician.[4–6] The patients who are affected by these multiple adenomas may present a variable clinical picture. For a number of years only one endocrine disorder may manifest itself, even if anatomically all three endocrine glands are abnormal. With further progress of the disease a complicated mosaic of endocrine symptoms may appear. Anterior pituitary failure or acromegaly as well as hyperparathyroidism and hyperinsulinism have been observed singly or in various combinations. This complex clinical pathologic picture has now been seen so often that the possibility of mere coincidence can be dismissed. There can be no question that we are dealing here with a nosologic entity which has a common pathogenesis, in other words with a syndrome. Since tumors of the anterior pituitary gland or parathyroid glands or islet cells cannot, so far as is known, themselves induce the development of analogous tumors in the two other endocrine glands in question, the three main glandular lesions of the syndrome have to be looked upon as the coordinated effects of a common cause. For this reason the developmental nature of the syndrome appears to be plausible, with a genetic disturbance as the most likely ultimate cause. Proof of the genetic character of a disease lies in its familial occurrence. So far no familial occurrence of adenomatosis of endocrine glands has been reported although about twenty cases have been published.†

† During the preparation of this paper two cases were reported which occurred in father and daughter.[14]

This presentation concerns not a single case but a family in which the syndrome of multiple endocrine adenomas has occurred in several members and in two generations. Two of these patients, Mrs. W. B. and Miss F. S., were hospitalized and their history is reported in detail. Two other sisters, the Misses C. P. and O. P., were seen only as outpatients. All these cases are described in the time sequence in which they were observed. The pathologic findings of these patients show certain common features which have not been stressed in the literature and which are discussed here. Certain lesions in non-endocrine organs, which are frequently found in the same patients, may also belong to the syndrome.

CASE REPORTS

Mrs. W. B., a married nurse of thirty-five years, was first seen on January 30, 1947. Her history is as follows: The patient had never been seriously ill until her twenty-eighth year when her menses, which had occurred regularly since her twelfth year, stopped suddenly. Two years later skull films were taken and these disclosed the presence of a small endosellar tumor. There were no headaches, no visual disturbances and no change in weight. At that time a fasting blood sugar of 60 and one of 47 mg. per cent were found. One year later the patient developed peculiar attacks which were strongly suggestive of spontaneous hypoglycemia. The patient had attacks of stupor during which she could not be sure whether activity around her was real or imagined, although she did not lose consciousness entirely and afterwards remembered vaguely what had happened. In these attacks the patient often made coordinated but purposeless movements like crossing her feet, twisting her hands or taking off her shoes. On rare occasions the

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patient fell to the floor during the attacks but there were never any generalized convulsions. Her face was flushed during an attack but there was no abnormal perspiration. The attacks always occurred either before breakfast or, especially if the patient had skipped her afternoon meal, before dinner, and they subsided promptly upon eating. They sometimes ended spontaneously after about an hour. For two years the attacks occurred several times a month but in the third year they became more frequent although their character remained the same. At about the same time the patient's personality changed. She became shy and withdrawn and spoke very little.

Physical examination showed an undersized woman who was well nourished and appeared healthy. Her skin and the distribution of hair were normal. The blood pressure was 114/80.

In view of the history of amenorrhea and the finding of sellar enlargement, the diagnosis of a chromophobic adenoma of the pituitary with hypopituitarism was made; spontaneous hypoglycemia was strongly suspected. The origin of the latter, however, was not clear when the patient was seen for the first time. Although the patient was advised to return for another examination within a few days, she did not return until seven months later. She then reported that the attacks of hypoglycemia had completely disappeared for about two months upon frequent sugar intake. The attacks subsequently recurred, however, first at intervals of several days and finally almost every day. The patient's mental condition had deteriorated further and she had been forced to give up her position.

The patient was now admitted for study. Her physical examination showed that she had gained 8 pounds in weight since the time she had been put upon a diet rich in carbohydrates. There were no abnormal physical findings. Gynecologic examination showed a very small uterus but no other abnormalities. The eye findings were normal. Skull films showed no further decrease in the size of the sella turcica. A laboratory work-up showed a basal metabolism rate of −18 per cent, a serum cholesterol of 180 mg. per cent and a radio-iodine uptake of 32.4 per cent. The urinary 17-ketosteroid excretion was 5.8 mg./24 hours. The serum sodium was 138 mEq. The serum calcium was 11.9 mg. per cent on two occasions. The serum phosphorus was 3.5 mg. per cent; the serum alkaline phosphatase was 3.2 Bodansky units. The fasting blood sugar ranged from 16 to 40 mg. per cent and one blood sugar taken soon after a lunch was 51 mg. per cent. A glucose tolerance test with 100 gm. of glucose orally gave the following results:

	Per cent
Fasting	16 mg.
30 minutes	95 mg.
60 minutes	101 mg.
120 minutes	81 mg.
180 minutes	69 mg.

An insulin tolerance test, with 6 units of regular insulin intravenously, gave the following results:

	Per cent
Fasting	33 mg.
After insulin:	
20 minutes	30 mg.
35 minutes	17 mg.
45 minutes	17 mg.
60 minutes	16 mg.
90 minutes	25 mg.
120 minutes	21 mg.

The patient developed twitching of the lips twenty minutes after the injection of insulin, at a time when the blood sugar had dropped to 30 mg. per cent. She was then given one glass of orange juice which led to the disappearance of twitching without raising the blood sugar appreciably. Only on this occasion and one other time when her blood sugar was documented at 27 mg. per cent did the patient present motor symptoms. At other times she showed only the peculiar frightened and withdrawn behavior described above.

On the basis of the clinical and laboratory findings, which tended to exclude impairment of adrenocortical and thyroid function, it appeared logical to conclude that the pituitary lesion was not the cause of hypoglycemia which, if due to anterior pituitary failure, occurs only in far advanced cases of Simmonds' syndrome. The presence of hyperinsulinism due to an independent tumor of the pancreatic islets therefore appeared probable and in view of the reported cases in the literature a diagnosis of adenomatosis of the pituitary, the islets and the parathyroids was made.

The patient was operated upon on September 15, 1947 (by Dr. Louis M. Rousselot). At exploration one large tumor was easily felt in the body of the pancreas and several smaller tumors were found in the tail. Since the presence of several tumors almost invariably means

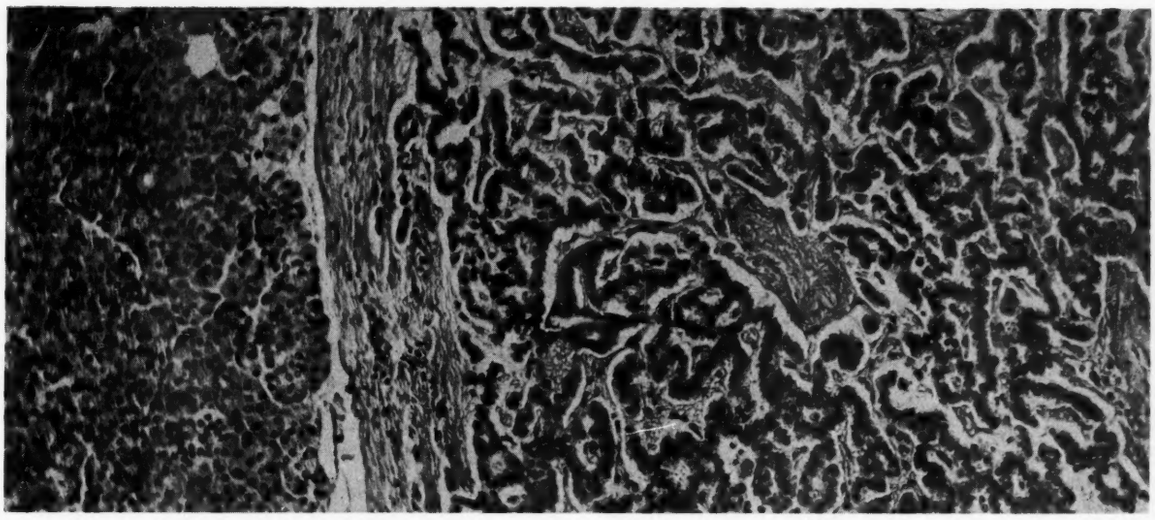

FIG. 1. Low power photomicrograph showing encapsulated tumor of ribbon type with some fibrosis. At the left is a rim of pancreatic tissue. (Courtesy of Dr. Virginia Kneeland Frantz.)

adenomatosis of the Langerhans islets—which had been expected preoperatively—a subtotal pancreatectomy was performed. The presenting tumor was oval, circumscribed, measured 1.6 cm. in greatest dimension and had a narrow rim of pancreatic tissue attached. The cut surface was pink with irregular scattered areas of orange softening. In the rim of pancreatic tissue attached to the tumor a small violaceous area was seen, a finding that further suggested the possibility of adenomatosis.

Microscopic examination showed nine separate tumors composed of cells resembling the islet cells in the adjacent pancreatic tissue. Some tumors were completely encapsulated, in others the capsule was ill defined. Many of the tumors contained scattered ducts, and in most of them there was a striking "ribbon" arrangement of cells. (Fig. 1.) No blood vessel invasion was found. Some tumors showed degeneration and fibrosis. In the adjacent pancreatic tissue there were numerous poorly defined, often enlarged islets. It is obvious that these findings are quite different from those of the more common isolated adenomas of the islands of Langerhans. The changes in the pancreas resemble however in every respect those in the other reported cases of the syndrome.

The patient's metabolic response to resection of the pancreas was most gratifying. There have been no hypoglycemic attacks since the operation and her mental condition became completely normal. Fasting blood sugar, calcium, phosphorus and alkaline phosphatase have been determined several times during the past few years and were found to be normal. Bone x-rays have shown no definite signs of abnormality.

The patient's family history was as follows: Her father and mother were born in Italy. They were not blood relatives. The mother had died at the age of sixty. She had had a cerebrovascular accident, was hypertensive and had had a gastric resection for cancer of the stomach at the age of fifty-eight. There was no autopsy. She had never suffered from symptoms of an endocrine disturbance. She had had nine children. The father, Mr. V. P., whose history was obtained in 1952 through the courtesy of Dr. Charles T. Olcott of Cornell University, had had a gastroenterostomy performed for a peptic ulcer in 1914. He died in 1922 of acute peritonitis. No endocrine anomalies had been observed during his lifetime. At autopsy, which was limited to the abdomen, an abscess was found in the gastrocolic ligament, due to perforation of the ulcer. In addition there were the following interesting findings: On top of the head of the pancreas a firm white nodule was seen, 5 mm. long, and the mucosa of the duodenum was riddled with innumerable white plaques, ranging from a few mm. to 1 cm. in diameter; these projected slightly over the surrounding mucosal level. The microscopic description reads as follows: "The pancreatic nodule gives the impression of a histological malformation—it would have been a relative overdevelopment of the anlage of the Langerhans islands in the center of the pancreatic lobule. The structure of the pancreatic nodule and of the submucosal nodules in the duodenum was the same."

Mrs. W. B. was the third of seven daughters. Two other children, both boys, had died in early infancy. When the patient was seen by us it was already known that the oldest sister, Miss C. P., the fourth sister, Miss O. P., and the fifth sister, Mrs. F. S., were suffering from pituitary tumors. The second sister and two younger sisters were in good health. As far as was known, neither grandparent on the paternal or maternal side of the family had had any unusual disease.

Miss C. P., the oldest sister, aged thirty-nine, was unmarried. Her history revealed that she had never menstruated. At the age of twenty she developed headaches and skull x-rays showed an enlarged sella turcica. The diagnosis of a chromophobic adenoma had been made and the patient had received three series of x-ray treatments to the pituitary. There were no ocular symptoms. At the age of thirty-eight the patient had developed attacks of disorientation in the early morning during which she wandered aimlessly about in her apartment and which subsided promptly if she drank a glass of fruit juice. There were no convulsive seizures. On physical examination the patient appeared undersized. Her hair distribution was normal. The breasts were small. The following fasting blood sugars were obtained: October, 1947: 51 mg. per cent; April, 1948: 80 mg. per cent; November, 1949: 66 mg. per cent; one serum calcium in March, 1951, was 11.5 mg. per cent. At the age of forty-three the patient developed post-prandial epigastric pain and a gastrointestinal series revealed the presence of a duodenal ulcer.

Miss O. P., aged thirty-two, was also unmarried. The patient's menstruation had begun at thirteen years and remained normal until her twenty-first year when it stopped. A pituitary tumor presumed to be a chromophobic adenoma had been found at the age of twenty-nine by skull x-rays. There was no history of hypoglycemic attacks. On physical examination the patient appeared normal. At the age of thirty-seven the patient developed epigastric pain and a duodenal ulcer was found by x-ray.

Mrs. F. S. was a thirty-four year old married woman. At the time of her hospitalization the history was as follows: She had had a normal childhood and adolescence. The vision in the left eye had been poor since childhood. Menarche was at eleven but her periods had ceased suddenly at twenty-one. She was married at twenty-three. Her weight then was 95 pounds. It had increased steadily to 153 pounds. When she was twenty-six her hands, feet and jaw gradually enlarged with separation of the teeth. At the same time she suffered attacks of severe left-sided retro-orbital headache. A pituitary tumor had been found on x-ray examination at the age of twenty-seven and the patient had received radiotherapy to the pituitary with temporary relief of the headaches. The acromegalic changes did not progress after the age of thirty. Eighteen months before the hospital admission the patient noted that if she was late for meals she felt somewhat weak and dizzy. During the eight months prior to admission there had been several episodes of mental clouding and incoherence before breakfast which were almost immediately relieved by drinking orange juice. During one of these attacks the blood sugar was found to be 32 mg. per cent. For a number of years the patient had had daily vomiting for periods lasting several months. There was no abdominal pain at any time. A gastrointestinal series taken elsewhere had been interpreted as negative. On physical examination the patient was obese and showed moderately severe acromegaly. Her blood pressure was 100/70. There was paralysis of the left internal rectus muscle and optic nerve atrophy in the left eye. The vision in the left eye was 5/200ths but normal in the right. The patient's fasting blood sugar was 51 mg. per cent, calcium 12 mg. per cent, phosphorus 2.7 mg. per cent, alkaline phosphatase 2.9 Bodansky units. Skull x-rays showed the same enlargement of the sella turcica as one and one-half years before, with generalized osteoporosis of the skull. The diagnosis of eosinophilic adenoma of the pituitary and of adenomatosis of the pancreatic islets and of the parathyroid glands was made and the patient was explored on May 10, 1951 (Dr. William Barclay Parsons). A subtotal pancreatectomy was performed for multiple tumors of the pancreas.

A few days postoperatively the patient had a very severe gastrointestinal hemorrhage from which she recovered. The blood sugars were all within normal limits after operation. Two months later, after a very stormy postoperative course, the patient was discharged from the hospital. She then developed abdominal pain and a mass was felt in the epigastrium. A gastrointestinal series taken in September, 1951, showed a gastric ulcer on the lesser curvature. On October 31, 1951, the patient underwent a partial gastrectomy with partial excision of a pseudocyst of the pancreas which

had formed in the area of the previous operation. The patient was discharged after three weeks but was readmitted eight days later with signs of acute peritonitis. On December 5th a third laparotomy was performed and perforation of a gastric ulcer, high on the anterior wall, was found and repaired. The patient ran a septic temperature after the operation and died one month later.

The findings in the specimen obtained at the first and second operations were as follows: The pancreas measured 15 by 4 by 1.5 cm. and lobular architecture was obvious. The serosa was pink and glistening. Embedded in the superior edge of the mid-portion of the pancreas was a soft, spongy, hemorrhagic-appearing mass measuring 4 cm. in diameter. This mass was easily freed from the surrounding tissue down to a narrow vascular pedicle which passed into the pancreas. On section of the mass several areas of slight firmness were noted. On serial section of the pancreas four other definite nodules were found deep in the gland. Three of these were situated in the proximal 5 to 6 cm. while the fourth lay near the tail. The nodules measured from 0.7 to 1.2 cm. in diameter. On microscopic examination, in addition to the five nodules noted on gross examination, thirteen additional adenomas were found. The adenomas varied in size from a part of a low power microscopic field to 3 cm. in diameter. The pattern of these characteristic adenomatous masses was one of ribbon-like cords composed of one or two rows of cells with a pinkish staining cytoplasm and large ovoid to spheroid basophilic nuclei containing numerous granules. Varying degrees of fibrosis and hyalinization were noted. In some nodules there was a small amount of delicate fibrous tissue separating the ribbons, while in others there was almost complete replacement of the nodule by dense hyaline fibrous tissue. Hemorrhage was noted in two nodules. The largest nodule showed clumps, strands and ribbons of cells separated by areas of hemorrhage and early fibrosis. A well developed capsule was noted. The smaller nodules, however, showed no encapsulation and in some areas there was extension of tumor cells into the surrounding pancreatic parenchyma. Gomori stain revealed cells with greyish-green cytoplasm containing fine pinkish granules and brilliant red nuclei with red granules. Many normal and smaller than normal islets were seen scattered throughout the specimen. The rest of the pancreas consisted of apparently normal acini with areas of fatty infiltration.

In that part of the duodenum which was removed at gastrectomy three small infiltrating tumors were found which were composed of sheets and anastomosing cords of polygonal cells. They were interpreted as islet cell adenomas originating in heterotopic pancreatic tissue although no acinar pancreatic structure was seen.

At postmortem a localized fibrinopurulent peritonitis secondary to perforated ulcer of the stomach was found. The findings in the skull were as follows: The sella turcica was distended by a mass of tumor tissue which arose from the pituitary. The gland was not separately distinguishable. The tumor measured approximately 3 cm. from side to side, 2 cm. anteroposteriorly and approximately 2 cm. dorsoventrally. It extended somewhat asymmetrically to the left of the midline as it rose out of the sella for a distance of approximately 6 to 8 mm. In the superior sella there were three poorly demarcated nodules in the tumor, the largest of which measured roughly 1.4 cm. anteroposteriorly and 1.2 cm. transversely. The tumor was covered on its dorsal aspect by a thin greyish capsule. The sella turcica measured 2.2 cm. from side to side and 1.8 cm. anteroposteriorly. The tumor had not penetrated into the sphenoid or ethmoid sinuses. The left optic nerve appeared to be slightly compressed from below upward and this flattening extended for a short distance into the optic chiasm. The right optic nerve was normal. Microscopically, the tumor was a highly cellular neoplasm which was quite vascular. In the highly cellular zone the tissue was traversed by a great many capillaries and had the architecture of the anterior lobe. The cells, however, were nearly all of the same size. There were occasional small cells with scanty cytoplasm. Some larger elements had coarsely granular cytoplasm. In most zones, however, there were dense, homogeneous bands of poorly cellular connective tissue which separated tumor cell islands of various size. The cells were essentially like those described above. On azocarmine stain many of the tumor cells contained eosinophilic granules. Some of the small elements contained no specific granules. The diagnosis of the pituitary tumor was mixed eosinophilic and chromophobic adenoma (Dr. Abner Wolf).

The parathyroids were markedly enlarged, soft and darker colored than usual, exhibiting a

yellowish-red tinge. On microscopic section, they were found to be composed of anastomosing cords of cells, most of them having a bluish-purple cytoplasm and round dark nuclei. The adenomatoid hyperplastic glands were highly vascular. The largest parathyroid showed areas with increased connective tissue bands. The thyroid was normal; it contained one small adenoma. The right adrenal was normal. The left adrenal contained a roundish nodule, measuring 1 by 0.5 cm. in diameter, consisting of columns of cortical cells resembling those of the zona fasciculata. This hyperplastic area was fairly vascular. There was no marked pleomorphism of the tumor cells. The ovaries were smaller than usual. They contained several dilated cyst follicles. Few primordial follicles were seen. Otherwise, the pelvic organs were grossly normal. Bone marrow from a vertebral body and a rib showed that the bony spicules were somewhat thinner than usual. There was, however, no osteoblastic overactivity. In the kidneys there were calcium deposits in the epithelium of several tubules. These tubules showed atrophy and necrosis with reactive fibrosis around them. No calculi were found.

The abnormalities presented by the five members of the family exhibiting the adenomatosis syndrome are summarized in Table I.

TABLE I

FINDINGS IN FIVE CASES OF ADENOMATOSIS OF ENDOCRINE GLANDS

Case	Age and Sex	Pituitary	Parathyroids	Pancreatic Islets	Gonads	Gastro-intestinal Tract	Anatomical Data
V. P.	41, M	.....	.....	.....	.....	Gastric ulcer	Islet cell adenoma of pancreas; islet cell adenomas of duodenum
C. P.	39, F	X-ray: intrasellar tumor	Serum calcium: 11.5 mg. %	Hyperinsulinism	Primary amenorrhea	Duodenal ulcer	.....
W. B.	35, F	X-ray: intrasellar tumor	Serum calcium: 11.9 mg. %	Hyperinsulinism	Secondary amenorrhea	.....	Adenomatosis of pancreatic islets
F. S.	34, F	X-ray: intrasellar tumor; acromegaly	Serum calcium: 12 mg. %	Hyperinsulinism	Secondary amenorrhea	Gastric ulcer	Mixed eosinophilic and chromophobic adenoma of pituitary; adenomatous hyperplasia of all 4 parathyroids; adenomatosis of pancreatic islets; islet cell tumors of duodenum
O. P.	32, F	X-ray: intrasellar tumor	.....	.....	Secondary amenorrhea	Duodenal ulcer	.....

COMMENT

The clinical course of our four patients, as observed over a period of about ten years, serves well to illustrate the various aspects of the syndrome of adenomatosis of endocrine glands. The patient O. P. has so far shown only signs of a pituitary tumor with gonadal failure and there can hardly be any doubt that her pituitary tumor is a chromophobic adenoma. The patients W. B. and C. P. present a picture very similar to that of their sister O. P. in respect to the symptomatology of the pituitary tumor, but in both these patients spontaneous hypoglycemia due to hyperinsulinism eventually was added to the symptoms of anterior pituitary failure. The main difference in the clinical course of the patients W. B. and C. P. was that C. P. has never menstruated, which probably signifies that in her case the onset of anterior pituitary failure began before puberty, while W. B. menstruated until the age of twenty-eight. It is obvious that there is no parallelism between the degree of symptoms referable to the various endocrine tumors since the hypoglycemic symptoms were of much less severity in case C. P. than in case W. B. In case F. S. the original symptoms were also those of a chromophobic pituitary tumor and in no way different from those of her sisters. It was

only several years after the menses had ceased that acromegaly began, which suggested that the patient's adenoma had then become eosinophilic. In contrast to several cases in the literature, in which generalized osteitis cystica or nephrolithiasis appeared as a result of hyperparathyroidism (Hadfield and Rogers, case 2; Kalbfleisch; Shelburne and McLaughlin; Underdahl et al., cases 4 and 8), no signs of this disease have been seen as yet in the group studied here, although moderate hypercalcemia was found on occasion in patients W. B., C. P. and F. S.; moderate in case F. S. despite the pathologic finding of adenomatosis of all four of the parathyroids. Absence of signs of hyperparathyroidism despite the presence of several parathyroid tumors has also been mentioned in the literature (Underdahl et al., Cases 1, 2, 3 and 5).

Occurrence of the syndrome of adenomatosis of endocrine glands in several members of a family warrants a discussion of its pathogenesis. Such a familial occurrence could be the result of pure coincidence, of environmental factors or of a genetic anomaly. The great rarity of the syndrome makes it more than improbable that chance played any role. Environmental factors, such as viral infections or abnormal intake of vitamins during pregnancy, likewise seem of little significance since the syndrome appeared in two generations and the four affected sisters were the product of four different pregnancies. One would therefore conclude that in the family reported here the adenomatosis was probably of genetic origin. Available data make it possible to analyze the type of inheritance.

It seems likely that, as in other hereditary syndromes, a single gene is responsible for its development. This hypothesis is strengthened by the fact that in the patients observed by us, as well as those reported in the literature, the syndrome appears as a more or less complete entitity whereas one would expect to find patients with a limited expression of the syndrome if it were due to more than one hereditary factor. Although such a limited manifestation of the syndrome has seemingly taken place in patient O. P., who has not shown any symptoms of hypoglycemia, one cannot rule out the presence of an asymptomatic adenomatosis of the islands of Langerhans even in this patient since hypoglycemia developed in her three sisters several years after the onset of hypopituitarism. Since the disease occurred in male and female patients in this family, an autosomal gene has to be postulated.

As regards the mode of inheritance, recessivity seems to be ruled out, since we cannot expect both parents, who were not consanguineous, to have carried the rare gene. That four of nine siblings were affected is another argument against recessive transmission. Dominant heredity, on the other hand, would be made probable by the appearance of the syndrome in two successive generations as well as by a high incidence among the siblings, both of which pertain here. Although the abnormal findings in patient V. P., the father of the affected daughters, were limited to the pancreatic islets—neither the pituitary nor the parathyroids could be examined—there is reason to suspect strongly that he suffered from the syndrome. The presence of tumors in the duodenal mucosa similar to those found in patient F. S., in addition to the tumor in the pancreas, seems to be of particular significance.

We have to conclude, then, that we are dealing here with an example of dominant hereditary transmission and it appears probable that one single autosomal gene is responsible for the whole pathologic picture. There seems to be a high degree of penetrance and expressivity of the gene. It appears likely, furthermore, that the anomalous gene originated as a mutation, possibly in one of the paternal grandparents of our patients and that it found its first phenotypic manifestation in Mr. V. P., their father. Analogous observations relating to the sudden appearance of mutations leading to the familial occurrence of a disease have been reported several times in the medical literature as, for instance, in chondrodystrophic dwarfism.[8]

It is likely that the isolated cases of the syndrome which have been published are of the same genetic origin as in the family we have studied since the clinical and pathologic findings in the isolated and familial cases are identical in every respect (as is true also of familial and newly arising cases of chondrodystrophy).

If there is a gene which causes the development of the syndrome, one might speculate about the existence of a normal allele, possibly in the same locus, which would be involved in the control of the regular development of the anterior pituitary, the parathyroids and the islands of Langerhans.* Since the three endocrine glands with which we are concerned start

* Objections have been raised lately to such an indirect proof of the existence of a normal gene (Goldschmidt).

their development at about the same embryologic stage, the idea of a common control of their growth in a normal and also in an abnormal direction seems permissible.

The concept of a genetic basis for the syndrome might be helpful in several other ways. The fact has already been mentioned that the structure of the islet cell adenomas in the syndrome differs from that seen in the more common single adenoma of the islet cells in a characteristic fashion: in the syndrome of endocrine adenomatosis many islets are hyperplastic, there are always several large adenomas present and the adenomas are characterized by a ribbon structure which one does not find in the single adenomas. If such a complex pathologic picture is found in one of the endocrine glands affected by the abnormal gene, one might anticipate the existence of analogous anomalies in other endocrine glands. As far as the parathyroids are concerned, this seems to be the case. In contrast to what is seen in the usual case of primary hyperparathyroidism the parathyroids show, in many cases, involvement of all the four glands; furthermore, while in primary hyperparathyroidism there are as a rule single adenomas, often consisting of one cell type, in the reported cases of the syndrome of adenomatosis a true adenomatous hyperplasia of the glands is present in which the adenomas are of varying cell type.[2–6] As regards the tumors of the anterior pituitary, there was no definite divergence from the macroscopic or microscopic picture of isolated pituitary adenomas although in a high percentage of cases the tumors—both chromophobic and eosinophilic adenomas—had grown far beyond the confines of the sella turcica (Claude and Baudouin; Molineus; Josefson; Lloyd; Cushing and Davidoff; Kalbfleisch; Underdahl et al., Cases 6, 10 and 11; patient F. S. of this report) and the term "malignant adenoma" was used by two authors.[3,4] The pathologic findings in the parathyroid glands and in the pancreas appear consistent with the opinion already expressed, that the syndrome is due to a factor other than that responsible for the development of isolated endocrine adenomas. This hypothesis is further strengthened by the fact that isolated adenomas of the anterior pituitary, of the parathyroids or of the islands of Langerhans occur only very seldom in several members of a family.[15] This in itself makes a genetic origin of these isolated adenomas improbable.

In this group of cases there is another not uncommon finding which deserves attention. In a very high percentage of these patients chronic gastric and duodenal ulcers were found. Among the five patients who belong to the family here reported there were two instances of gastric ulcer and two of duodenal ulcer. Among the eight cases reported by Underdahl and co-workers there were three patients with duodenal ulcers and one of these patients also had a gastric ulcer. Among fourteen other cases reported in the literature, there were three who suffered from duodenal ulcer, one of which perforated.[5,10,13] It seems very questionable that hypoglycemia could be responsible for development of the ulcers because in several patients no hypoglycemic attacks had occurred at the time the ulcer was found. This leads to the possibility that peptic ulcer may represent one more manifestation of the abnormal gene. The high incidence of peptic ulcer suggests that the penetrance of the gene for the formation of ulcer is not different from that for adenomatosis.

Another gastric anomaly which sometimes occurs concurrently with endocrine adenomatosis is diffuse gastric polyposis (Menetrier's disease). It was found in two of Underdahl's eight cases.

From the clinical point of view the correct classification of cases of hyperinsulinism and hyperparathyroidism is obviously of great importance. The operative procedure in a case of multiple adenomas of the islet cells, as well as in a case of multiple adenomas of the parathyroid, is different from that in a single adenoma of these glands. It is therefore necessary to keep the possibility of the presence of the syndrome in mind whenever a patient is seen who suffers from a tumor of either the anterior pituitary, the parathyroids or the islets of Langerhans. Fortunately, the recognition of patients who belong in the group of adenomatosis of endocrine glands is not difficult once the characteristic features of the syndrome are known. It is necessary, however, to remember that the symptomatology in such a case may for many years be limited to only one diseased gland.

SUMMARY

1. The syndrome of adenomatosis of the anterior pituitary, the parathyroids and the pancreatic islets was observed in a family in which the father and four of nine siblings were

affected. It is assumed that the syndrome in this family was caused by a dominant autosomal gene with a high degree of penetrance.

2. The gross and microscopic findings in the parathyroids and in the pancreas differ from those seen in the more common single adenomas. The therapeutic approach therefore has to be different in the two types of cases.

3. Peptic ulcers of the stomach and of the duodenum are frequently found in patients suffering from the syndrome of adenomatosis of the endocrine gland. This may be looked upon as another manifestation of the abnormal gene.

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Pathologic Changes in Normal Human Thyroid Tissue Following Large Doses of I-131*

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THERAPEUTIC doses of I-131 have been given to patients for a variety of pathologic conditions. This isotope has been used for the treatment of hyperthyroidism; for the destruction of normal thyroid tissue in preparation for radioiodine treatment of patients with carcinoma of the thyroid; and for the therapy of lesions of carcinoma of the thyroid. It has been used also in certain cases of intractable heart disease to lower the basal metabolic rate.

There is little agreement on the amount of I-131 needed to destroy completely a normal human thyroid gland. Dobyns and Maloof[1] used radioiodine to produce this effect in five patients with thyroid carcinoma in whom only a biopsy had been done previously. They found the method unsatisfactory because relatively large doses were needed and because the initial dose was not always adequate for complete destruction of the normal gland. They reported that after most of the thyroid had been resected it was relatively easy to inactivate the remaining thyroid tissue with 25 to 50 mc. of I-131. They believe that the hypertrophied remnants may be especially vulnerable to radiation. Rawson and Trunnel[2] state that normal thyroid tissue remaining after incomplete surgical removal may be destroyed by giving I-131 in doses which permit localization of 600 to 700 μc. per gram estimated weight of tissue. We have also had the experience that small residual portions of thyroid remaining after subtotal thyroidectomy are relatively easily destroyed with I-131. Presumably these masses of tissue are not so small in relation to the beta penetration that the effectiveness of radiation is greatly diminished by lack of cross-fire effect.

Blumgart and associates[3] report treatment of thirty euthyroid cardiac patients with I-131. In their experience myxedema was finally produced in each case in five weeks to six months but the total amount of I-131 needed for radiation thyroidectomy ranged from 25.5 to 200 mc. and as many as six individual doses were sometimes needed. Their largest single dose was 51 mc.

The pathologic changes produced by radioiodine have been studied quite extensively in experimental animals.[4–6] In rats and mice the changes produced are different from those seen in the human in two important respects. One of these is that the sequence of morphologic evidences of radiation effect occurs much more rapidly than in the human. For example, in the 250 gm. rat doses of 525 μc. and higher produce massive destruction of the thyroid within forty-eight hours.[6] In addition, the size of the organs in small animals in relation to the path of the β particle is such that changes are more commonly produced in the adjoining structures, especially the parathyroids and trachea.[4]

Reports of the effect of radiation of the normal human thyroid with x-ray and radium[7] have tended to emphasize the relative radioresistance of this organ.

Several reports have appeared on the histologic effects of radioiodine in patients with hyperthyroidism. Recently, Vickerey[8] described the histologic effects of therapeutic doses of I-131 found in a series of twenty-five patients whose thyroids were biopsied at varying intervals up to eight years following treatment. This study emphasized the late effects of doses given for only partial destruction of the thyroid gland; doses which were much smaller than those usually used for the treatment of carcinoma.

Information on the changes produced by I-131 in the normal human thyroid has been obtained in patients treated for carcinoma and

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heart disease. Godwin and associates[9] in their postmortem study of nine patients who had received I-131 for the treatment of thyroid carcinoma contribute little information concerning the changes produced by the isotope in the normal thyroid tissue. Various histologic changes in the neoplasm, ascribed to the effects of I-131, are emphasized. The most specific information they give on this problem is in reference to their patient (Case 8) who received a total of 820 mc. of I-131 during a two-year period: "Microscopic study of the thyroid tissue showed fibrosis with many foci of viable follicular and alveolar carcinoma . . . the remainder of the gland showed nodular goitre without significant cellular alterations." In a later reference to this case they state: "In one patient (Case 8) to whom 820 mc. of I-131 had been administered, there is no histological change in the remaining (non-cancerous) thyroid tissue." This patient apparently demonstrated unusual persistence of normal thyroid tissue after a very large total dose of radioiodine.

A very complete and informative report has been presented recently by Freedberg, Kurland and Blumgart.[10] This study was based upon autopsy examinations in a group of patients treated with I-131 for severe and intractable heart disease and one patient with thyroid carcinoma. Detailed histologic descriptions of the thyroid glands and adjoining structures are reported. Our observations are very similar to those of Freedberg and associates; the present study confirms their findings and presents additional data on thyroid glands studied at short intervals after administration of the isotope.

MATERIAL AND METHODS

Table 1 summarizes all of the pertinent data upon which the present report is based. The patients studied were hospitalized while being treated with radioiodine. All except one (Case 2) had carcinoma of the thyroid which could not be treated by surgical methods alone. The single exception was a patient with widespread metastatic malignant melanoma. The I-131 was given to this patient in order to obtain information concerning the concentration of I-131 in the lesions of malignant melanoma.

Three of the patients (Cases 7, 8 and 10) were given large doses of radioiodine for the purpose of producing thyroidectomy by radiation alone. One of these was subsequently studied at autopsy. The other two patients were subjected to surgical thyroidectomy shortly after the large dose had been given because re-evaluation of the clinical situation indicated the need for more rapid removal of normal thyroid tissue. (One of these patients (Case 8) was the only one in the series with metastases which concentrated significant amounts of radioiodine.) The additional six patients make up a group studied during the last eighteen months in which a large dose of iodine was given with the deliberate intention of following this with surgical thyroidectomy. This procedure was considered justifiable as a means of obtaining the histologic information reported in this article. It was realized that such a plan of treatment might be less desirable than surgical thyroidectomy alone as a preliminary phase of the I-131 treatment of thyroid carcinoma, since the initial dose of radioiodine gives unnecessary radiation to the bone marrow in a patient who may later need large amounts of I-131 for treatment. Furthermore, the initial small amounts of I-131 deposited in the metastases are ineffectual and may tend to decrease the capacity of the tumor tissue to concentrate therapeutic doses of radioiodine given later. Because of these objections we did not include in this study patients with well differentiated colloid-producing lesions or patients who initially showed concentration of I-131 in metastases.

The histologic observations in the present report are limited to the non-neoplastic thyroid tissue without consideration of radiation changes in the tumors. In the several cases which also contained adenomatous or nodular goiter the observations are on the thyroid tissue not involved in the adenomatous process, since frequently in such lesions degenerative changes, not related to radiation, are encountered. Gross and microscopic autoradiograms were prepared from most specimens. Formalin-fixed tissues were studied by routine histologic methods employing hematoxylin and eosin stains, phosphotungstic acid hematoxylin stains and fat stains.

RESULTS

The following are gross and microscopic descriptions of the thyroid glands in this series:

Case 1 (No. 110115). C. M., a seventy-five year old woman, was given a test dose of 1 mc. of I-131 nineteen days before thyroidectomy, and a larger dose of 28 mc. two days prior to surgery. The thyroid showed, in addition to a mass of neoplasm, a multiple nodular goiter of

TABLE 1

Case No., Initials, Hosp. No., Sex and Age	Diagnosis	Amount of Thyroid Tissue Previously Removed	Previous X-ray Therapy	Doses I-131 (Mc.)	Time Intervals to Death or Operations (Days)	Per Cent Excreted in Four Days	μc./Gm. in Thyroid Corrected to t^0 for Last Dose	Essential Histologic Changes
Case 1 C. M. No. 110115 Female 75 yr.	Mixed type undifferentiated carcinoma of thyroid	None	None	1. 28.	19 2	75 77.2 (2 days)	144.1 Some tumor 81.3 in these 12.1 specimens 11.4 19.3 Av. 53.6	None
Case 2 W. D. No. 110058 Male 37 yr.	Malignant melanoma; no thyroid lesion	None	None	57.5	3	55 (3 days)	424.7	None
Case 3 M. C. No. 110119 Female 16 yr.	Adenocarcinoma of thyroid	Part of one lobe	None	28.	3	64.4 (3 days)	95.4	Minimal
Case 4 J. H. No. 210194 Male 38 yr.	Alveolar carcinoma of thyroid	None	None	72.7	5	83.1	231.4 713.7 Av. 472.6	Minimal
Case 5 T. M. No. 110122 Male 43 yr.	Papillary carcinoma of thyroid	One lobe and isthmus	Therapy during a 39-day period; concluded 50 days before thyroidectomy; tumor dose in region of thyroid estimated between 3,750 and 4,500 r	45.7	9	76.9	668.3 777.2 791.8 Av. 745.8	No definite effects
Case 6 L. D. No. 110131 Female 26 yr.	Alveolar and papillary carcinoma of thyroid	Part of one lobe	None	1.2 72.5	16 9	71.1 66.3	426.2 474.6 Av. 450.4	Patchy necrosis
Case 7 O. L. No. 110080 Male 63 yr.	Papillary carcinoma of thyroid	One lobe and isthmus	High dosage 7 yr. before given I-131	87.0	13	84	91.9 72.2 149.6 Av. 104.6	Massive hemorrhagic necrosis
Case 8 A. H. No. 110085 Female 46 yr.	Alveolar adenocarcinoma of thyroid	Part of one lobe	None	2. 79.5	27 16	46.4 61.1	160 89 244 Av. 164	No definite effects
Case 9 A. S. No. 210137 Female 60 yr.	Adenocarcinoma of thyroid Hürthle cell type	All of one lobe and part of the other	4,800 r 200 kv. 15 ma. ½ cu. 50 cm. Received 5 yr. before given I-131	1.1 74.0 (0.3) 0.3	49 42 8 1	63.8 67.7 67.3	.351 .988 Av. .670	Severe necrosis and lipoid degeneration
Case 10 A. W. No. 010025 Female 70 yr.	Alveolar carcinoma of thyroid	None	None	2. 47.4 3.0 47.0 114.0 84.2 297.6	122 108 73 69 33 10	78.5 37.4*	69.1	Fibrosis and regeneration

* Incomplete collection.

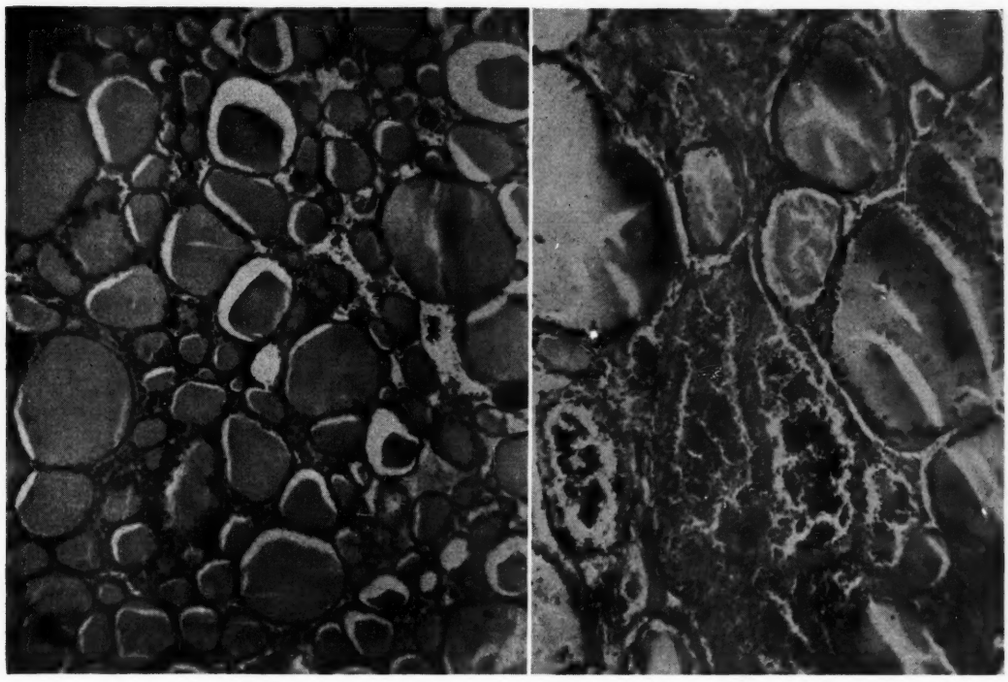

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FIG. 1. Case 1. No changes seen two days after 28 mc. of I-131. Hematoxylin and eosin stain, × 100.

FIG. 2. Case 3. This selected field shows an area with leukocytes infiltrating a follicle and the interstitial tissue. In most of the section, this abnormality was not seen. Hematoxylin and eosin stain, × 100.

the right lobe. There was also normal nonnodular thyroid tissue remaining. Microscopically the thyroid showed no alterations which could be attributed to radiation. (Fig. 1.)

CASE 2 (No. 110058). W. D., a thirty-seven year old man, was given 57.5 mc. of I-131 three days before death. The thyroid gland removed at autopsy was normal in appearance and weighed 13.7 gm. On microscopic study there were no effects which might be due to radiation. There was a variable appearing colloid, some of which had a granular appearance. Autoradiograms showed uptake of I-131 in an uneven pattern, localized irregularly to the regions of some follicles.

CASE 3 (No. 110119). M. C., a sixteen year old girl, received 28 mc. of I-131 three days prior to surgery. The left lobe of the thyroid, which was removed, measured 3 by 2 by 1.5 cm. and contained no nodular goiter. Histologically there was general preservation of thyroid structure. However, in certain scattered fields there was partial loss of the lining epithelium, and inflammatory cells were infiltrating these areas. Polymorphonuclear leukocytes and monocytes were seen in the colloid of some of the follicles, and some of the colloid itself showed a granular appearance. In a few places hemorrhage into the follicles had occurred. (Fig. 2.)

CASE 4 (No. 210194). J. H., a thirty-eight year old man, received 72.7 mc. of I-131 five days before surgical thyroidectomy. Only one-third to one-fourth of the thyroid gland had not been invaded by tumor. This thyroid tissue was normal on gross examination. Microscopic studies showed generally intact thyroid structure. Occasionally a follicle showed some disruption and contained a few leukocytes or erythrocytes. Foci of leukocytes and extravasations of erythrocytes were found in the interstitial tissue. Autoradiograms showed the usual uneven follicular pattern of I-131 uptake. (Fig. 3.)

CASE 5 (No. 110122). T. M., a forty-three year old man who had had a previous hemithyroidectomy, received 45.7 mc. of I-131 nine days before surgery. The remainder of the thyroid, removed at this time, weighed 10 gm. and showed no nodular goiter. The microscopic structure was unaltered except for a rare follicle showing lymphocytic infiltration and loss of epithelium. No distinct effect which could be attributed to radiation was found. Autoradiograms showed the usual uneven uptake of I-131.

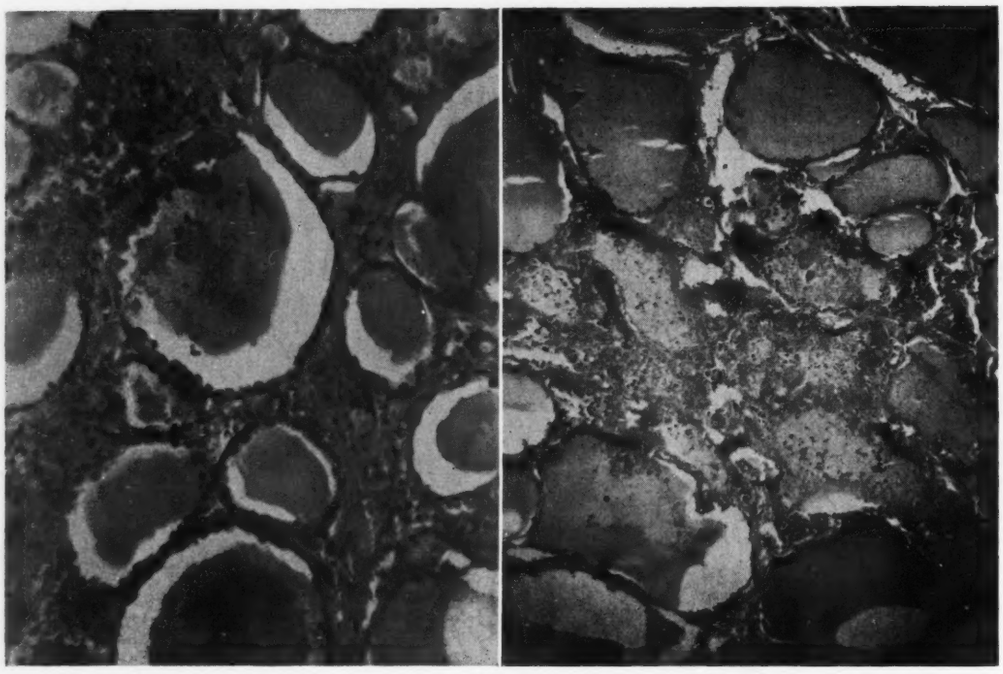

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FIG. 3. Case 4. Note minimal cellular infiltration of follicles and hemorrhages in some follicles. There appeared to be swelling and separation from the wall of some epithelial cells. Hematoxylin and eosin stain, × 200.

FIG. 4. Case 6. A group of necrotic follicles is shown. The epithelium is destroyed and only fragments of colloid persist. There are leukocytes, erythrocytes and fibrin in this area. This tissue had been removed nine days after 72.5 mc. of I-131. Hematoxylin and eosin stain, × 100.

CASE 6 (No. 110131). L. D., a twenty-six year old woman, had had a partial thyroidectomy previously. A test dose of 1.2 mc. of I-131 was given sixteen days before surgery. Nine days before operation 72.5 mc. of I-131 were given. All of the residual left lobe and approximately one-half of the right lobe of the thyroid were replaced by carcinoma. The residual thyroid tissue was normal on macroscopic study. Histologic examination revealed a general preservation of thyroid lobules and architecture. Most of the follicles were intact. However, small groups and single follicles showed degenerative changes and these varied in degree. In some of these there was complete necrosis with destruction of the epithelium and infiltration by inflammatory cells. Although there was some hemorrhage into isolated follicles, this was not a prominent feature. Microscopic autoradiograms showed normal patchy follicular distribution of I-131. (Fig. 4.)

CASE 7 (No. 110080). O. L., a sixty-three year old man, was given 87 mc. of I-131 thirteen days before surgery. The left lobe and isthmus of the thyroid had been removed earlier. The right lobe of the thyroid was dark red and hemorrhagic in appearance. It showed no nodular goiter. Histologically there was a massive hemorrhagic and coagulation necrosis of the thyroid gland. Hemorrhage had occurred into some of the follicles and throughout the interstitial tissue. The adjacent skeletal muscle also contained extravasated erythrocytes. Remnants of follicles could be seen, identifiable chiefly by the presence of colloid. Degenerating neutrophilic leukocytes were seen in many necrotic areas. Some small arterioles showed old obliteration of the lumens. Other vessels showed numerous leukocytes in the walls and in the adjacent tissue. In some areas near the edge of the gland coagulation necrosis was not as complete. In the very necrotic zones all vessel walls shared in the necrosis. In the less severely attacked sites, where remnants of histologic detail were better preserved, numerous eosinophils were found. The microscopic autoradiograms showed an uneven distribution similar to that seen in thyroid glands without necrosis. In spite of the advanced necrosis the I-131 remained localized to the pattern of the follicles. (Figs. 5 and 6.)

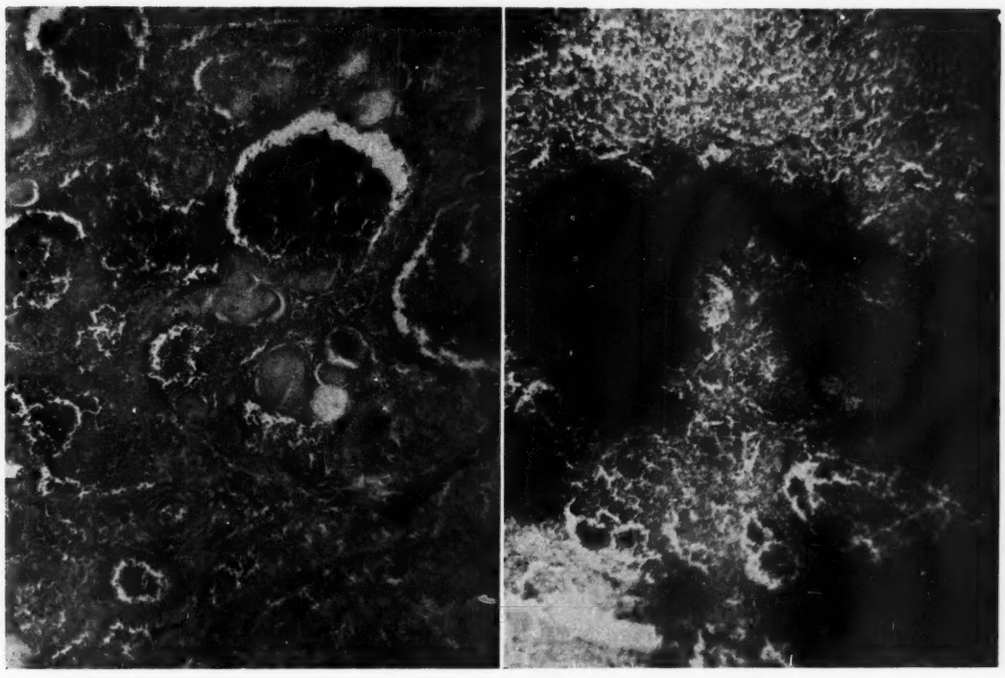

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FIG. 5. Case 7. Massive hemorrhagic necrosis of the thyroid gland thirteen days after 87 mc. of I-131. Colloid persists as round homogeneous areas. Hematoxylin and eosin stain, × 100.

FIG. 6. Case 7. Autoradiogram from a different area than Figure 5. No persistence of follicular distribution of I-131 in spite of complete necrosis. Hematoxylin and eosin stain, × 100.

CASE 8 (No. 110085). A. H., a forty-six year old woman, had had a partial thyroidectomy elsewhere. She was given a 2 mc. test dose of I-131 twenty-seven days before thyroidectomy and a large dose of 79.5 mc. of I-131 was given sixteen days before the operation. The thyroid contained adenomas and was normal in size although there was an increase in consistency. Microscopic study of non-adenomatous areas of thyroid gland revealed many macrofollicular acini alternating with smaller follicles. There was no inflammatory response and no breakdown of follicles. Some of the colloid was granular or vacuolated. Autoradiograms showed an uneven concentration of I-131 localized in the follicles. (Fig. 7.)

CASE 9 (No. 210137). A. S., a sixty year old woman, had undergone a partial thyroidectomy prior to admission. A test dose of 1.1 mc. of I-131 was given forty-nine days before thyroidectomy, and 74 mc. was administered forty-two days before the operation. The thyroid tissue was firm, pink and yellow-tan in color and weighed approximately 3 gm. There was no nodular goiter. On histologic examination severe coagulation necrosis of most of the thyroid tissue was found. Many follicles showed complete destruc-

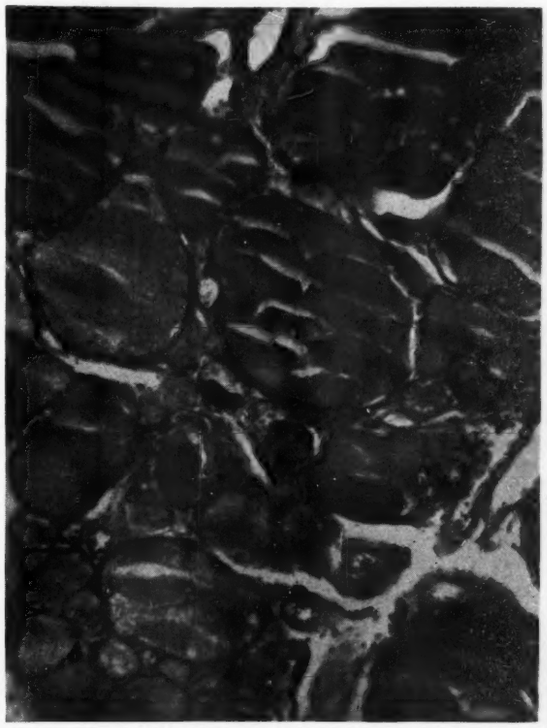

FIG. 7. Case 8. This tissue shows intact architecture, even though 79.5 mc. of I-131 had been given twenty-seven days earlier. Note granular appearance of colloid. Hematoxylin and eosin stain, × 100.

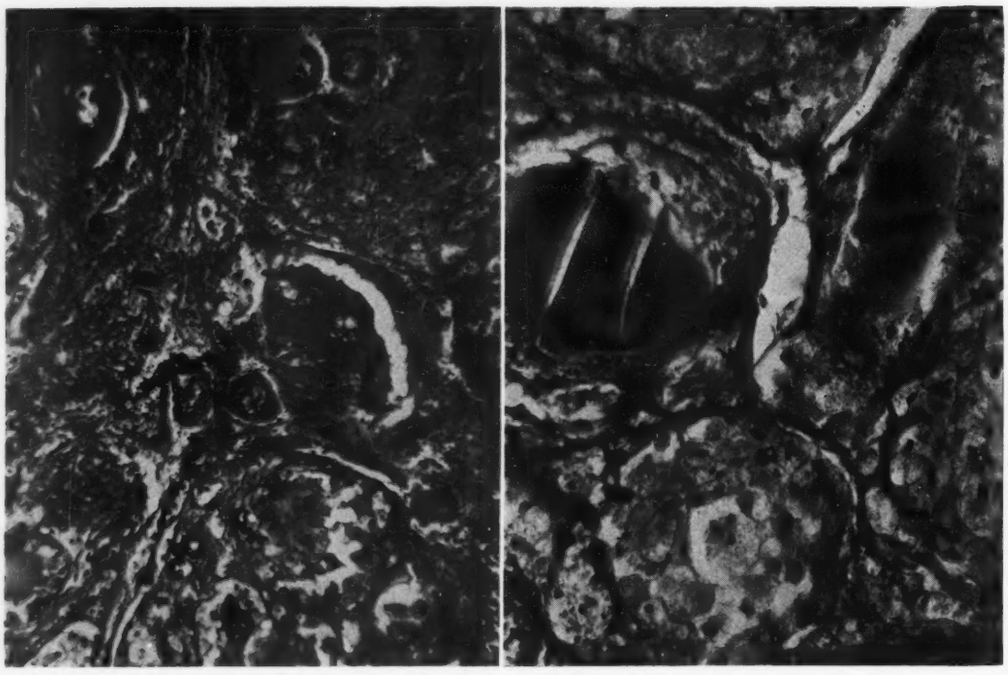

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FIG. 8. Case 9. Thyroidectomy forty-two days after 74 mc. of I-131. Extensive but not complete necrosis of the thyroid gland is shown. There is destruction of the epithelium and peculiar scalloped vacuolization of the colloid. The most striking feature is the presence of large foam cells at the site of some follicles and in the interstitial tissue. Hematoxylin and eosin stain, × 100.

FIG. 9. Case 9. Higher power view of Figure 8. PTAH stain, × 200.

tion while others retained colloid and follicle outlines. In the areas of moderately severe destruction it was still possible to recognize an indistinct follicular pattern. In slightly less damaged sites the colloid was still present and the follicular outlines were better preserved. Even here the epithelium had usually been destroyed. There were polymorphonuclear leukocytes scattered throughout, and some areas showed large foamy phagocytes. These gave a positive fat reaction with Oil Red-O. In many instances they were seen filling or partially occupying the sites of follicles. Hemorrhage into scattered follicles and some areas of stroma was also seen. Fibrin was deposited in heavy strands in some of the connective tissue trabeculae. In spite of the advanced destruction of the gland autoradiograms showed an uneven localization of I-131 in scattered follicles. (Figs. 8 and 9.)

CASE 10 (No. 010025). A. W., a seventy-nine year old woman, received six doses of I-131 totaling 297.6 mc. These doses were given at intervals between 122 days and ten days before death. At autopsy a 3 by 4 cm. portion of thyroid gland weighing 8.9 gm. was obtained. In addition to carcinoma, nodular goiter was present, interspersed with normal thyroid gland. Histologically the thyroid lobules in non-adenomatous portions were small and there was considerable increase in fibrous tissue trabeculae. Many of the follicles were unusually small. These microfollicular areas were arranged within wide bands of connective tissue. The rather solid acinar appearance in places simulated neoplasm on casual inspection. However, lack of anaplasia and the presence of distinct morphologic differences from the frank tumor in adjacent areas suggest that these areas represent non-neoplastic thyroid tissue. (Fig. 10.)

COMMENTS

It would be desirable to report accurately the radiation dosage for each thyroid gland studied in this series. Such calculations, utilizing the formulas of Marinelli, Quimby and Hine,[11] must be based upon factors which are variable and difficult to measure. One would have to know the volume of thyroid tissue, and this cannot be assumed to be the same initially that it is later at operation or autopsy, since radiation may produce swelling followed by atrophy. Another essential series of determinations are

those of the varying amounts of radioiodine in the thyroid gland during the period of radiation effect. There is gradual release of radioiodine from the thyroid after its initial concentration and, when large doses are given, there is tissue destruction which is associated with an enhanced outpouring of the isotope and a secondary rise in the blood radioactivity.[12] In the presence of functioning thyroid neoplasm there is still further difficulty in determining the amount of radioiodine in normal thyroid tissue, since urinary excretion studies reflect only the total amount retained and external counting studies may be unsatisfactory if some of the neoplasm is adjacent to normal thyroid tissue.

Valuable information can be obtained by measurement of the amount of radioiodine in the thyroid at frequent intervals, utilizing external counting technics. This has been done many times after tracer amounts but not often following large, necrotizing doses of the isotope. From such information one could derive an integrated dosage calculation more satisfactory than those which assume a uniform pattern of release of the isotope and which are based on a biologic half-life determined from short-term urinary excretion studies. Such external counting measurements are not available for the present series of cases, however.

In most of our cases information is available on the I-131 content of the thyroid tissue as determined by radioassay after operation or autopsy. This is not a sound basis for the calculation of radiation dosage, however, because of changes in the size of the gland, and especially because of the enhanced late outpouring of the isotope from the gland in cases in which destruction was especially severe. Thus it is not surprising that in our Case 9 the I-131 content of the thyroid was not high in relation to the dose given and the effects seen.

Even with all of the information possible by current methods, dosage calculations are handicapped by a serious and fundamental error. In these calculations it is assumed that the isotope is evenly distributed in the tissue while actually it is known to be very unevenly distributed, as is demonstrated by autoradiograms in the present series of cases. Another and probably less important cause of error derives from the variable volumes and irregular shapes of the thyroid tissue with resulting inaccuracies in dosage calculations.[13]

In general, calculated dosages of radiation

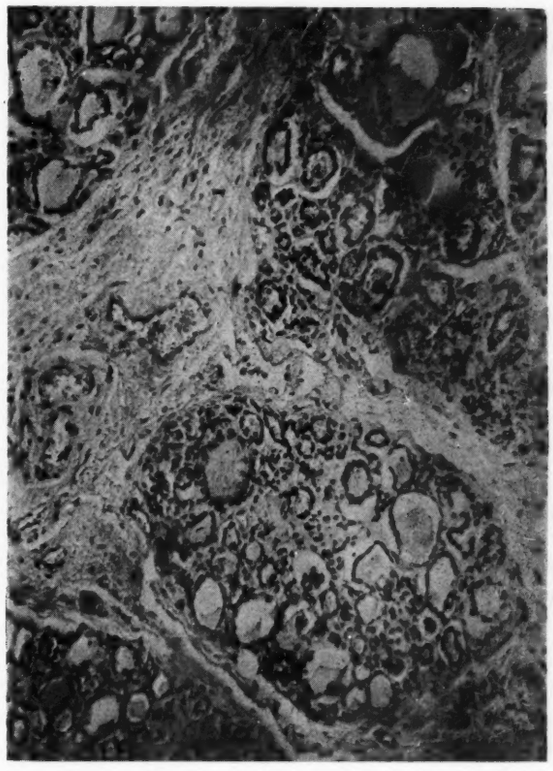

FIG. 10. Case 10. This patient received a total of 297.6 mc. of I-131 in six doses. In spite of this dosage follicles, many of them small, are present. The trabeculae are fibrosed. Hematoxylin and eosin stain, × 75.

from radioiodine are associated with biologic effects less severe than would be produced by supposedly equivalent doses of external gamma radiation. It is customary to explain this discrepancy on the basis of uneven distribution of the isotope, which results in relatively little irradiation in certain areas while much of the beta dose is ineffective because of concentration above necrotizing level in other zones.[14] Thus it is not surprising that complete and uniform necrosis does not occur even with large doses of radioiodine. However, this explanation seems less valid if one considers the failure to produce any necrosis, even in the areas of maximum effect, after doses which are smaller but which nevertheless would be considered rather high if produced by external gamma radiation.

In some of the cases of the present series the histologic specimens were obtained so soon that there was probably not time for morphologic evidences of radiation effect to develop, but this factor, too, seems inadequate to account for the discrepancies in biologic effect as related to dosage. Because of all of these difficulties and limitations we believe that dosage calculations

may be misleading and have not included them in the present report.

It is not possible on the basis of this series of cases to describe in detail the sequence of early histologic changes in normal human thyroid tissue following administration of radioiodine. Although we have examples at two, three, five, nine, thirteen, sixteen and forty-two days, the severity of the changes is determined by dosage and other unknown factors as well as by the time intervals.

Operative trauma from earlier thyroid surgery, as well as the damage produced during resection of the specimens described here, may account for some of the histologic changes seen. We are inclined to discount the importance of previous x-ray therapy, which had been received by three patients, because of the relatively long time intervals and low tissue dose as compared with that of the I-131.

It seems most satisfactory to consider the histologic changes in relation to the time interval after the dose of radioiodine. Cases 1 and 2 studied at two- and three-day intervals showed no alteration. However, in Case 3, also at a three-day interval, a few foci of leukocytes were found in widely scattered follicles. Slightly more pronounced changes were found in Case 4 removed at five days after a 72 mc. dose of I-131. These changes consisted of disruption of the epithelium of occasional follicles with a few leukocytes, erythrocytes, or both, in the follicles. In addition a few scattered foci of polymorphonuclear leukocytes and erythrocytes were found in the connective tissue stroma of the gland. The changes appear to represent patchy localized inflammatory response resulting from direct radiation damage, added to extravasations of red cells caused by injury to the small capillaries. At nine days, as shown by Case 6, some single follicles and small groups of follicles showed varying degrees of destruction. Occasionally there was complete necrosis with destruction of the epithelium and infiltration of inflammatory cells. Hemorrhage was not particularly prominent in this case. Case 5 was also studied at nine days after a large dose of iodine, and a higher concentration of I-131 was found in the gland at operation. However, there were no distinct alterations which could be attributed to radiation.

In contrast to the first six cases in which there was generally intact histologic architecture, the thyroid in Case 7 removed thirteen days after the dose of iodine was given, demonstrated a startling, widespread hemorrhagic necrosis. The autoradiograms showed persistence of the follicular pattern of iodine deposition. It seems clear that this does not represent the iodine-collecting ability of a necrotic thyroid but, rather, demonstrates the persistence of the initial pattern of deposition in spite of subsequent necrosis of the tissue.

In striking contrast with this picture of extensive destruction was Case 8, with a thyroidectomy sixteen days after the dose of I-131, a slightly longer interval than in the previous case. Approximately the same dose was given but in spite of a relatively high concentration of the isotope in the thyroid tissue there was no inflammatory response and no breakdown of follicles. This patient differed from others in the group in that she had massive metastases of functioning thyroid tumor. If it were not for the higher radioiodine concentration in the thyroid, one might be tempted to assume that the normal tissue was protected because of the diversion of a large portion of the isotope to the tumor. In Case 9 we were able to observe changes at a considerably later interval, forty-two days after the administration of 74 mc. of radioiodine. There were pronounced pathologic changes and certain features were quite different from those seen in the other cases. In addition to necrosis and inflammatory infiltrate many foam cells were present in the sites of follicles and in the stroma. Some of the foam cells are believed to be degenerating epithelial cells. This picture appears to represent incomplete destruction of the gland. The persistence of functional activity was demonstrated by the concentration in the thyroid of a significant quantity of a tracer dose given one day before thyroidectomy. Case 10 is difficult to interpret because of the multiple large doses at different intervals. Satisfactory excretion data are not available for this patient; assays made by external counting showed significant concentration of the isotope in the normal thyroid tissue. The histologic appearance is believed to represent radiation fibrosis with islands of small follicles which may be regenerative or persistent radioresistent thyroid tissue.

It is of some interest to note that the two patients (Cases 7 and 9) whose thyroids histologically showed the most severe radiation damage also displayed clinically the most severe inflammatory reaction with erythema, tenderness and edema in the region of the thyroid

following I-131. One patient (Case 9) required a tracheotomy because of tracheal compression produced by edema of tissues reacting to I-131 radiation; the other developed considerable pain and swelling adjacent to the thyroid.

Although accurate calculations of tissue dosage were not possible in the present series of cases, some consideration has been given to the extent of histologic damage in relation to dosage given, per cent retained, size of the gland, interval of radiation, and final radioactivity of tissue. It seems apparent that one cannot predict the degree of effect which will be produced by a certain dose of radioiodine, even when the per cent retention has been estimated from a previous tracer study. There is great variability in response and some patients are found to have functioning thyroid tissue even after large amounts of the isotope have been administered. This uncertainty of effect, along with other factors, emphasizes the desirability of surgical thyroidectomy rather than radiation destruction of the gland as the initial step in radioiodine therapy of thyroid carcinoma.

SUMMARY

1. The early histologic changes which occur in normal thyroid tissue after relatively large doses of I-131 are described on the basis of information obtained from ten patients.

2. Because of variations in size of the gland and differences in concentration and retention of the isotope, the radiation dose to the thyroid tissue is not closely correlated with the total dose administered.

3. Even when all of the factors known to influence tissue radiation dosage are considered, it is not possible to predict the biologic response on the basis of dosage.

4. The accomplishment of complete destruction of the thyroid by means of a single, safe dose of radioiodine is a rather uncertain procedure. In some cases large total doses have failed to cause complete necrosis of the gland.

Addendum: Since the submission of this manuscript another valuable contribution on this subject has appeared by Dobyns and associates.[15]

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Hemochromatosis and Transfusional Hemosiderosis*

A Clinical and Pathologic Study

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ALTHOUGH the clinical and morphologic features of hemochromatosis have been thoroughly investigated, a study to compare this disease with other conditions characterized by extensive deposition of hemosiderin in the body, such as transfusional hemosiderosis, has not been made. It was thought that such a study might clarify some of the problems of iron overload. Recently there have been several investigations on the pathogenic effects of hemosiderin which have occurred after repeated transfusions of blood. Occasionally the extent of cutaneous and visceral pigmentation which sometimes is associated with transfusional hemosiderosis has been comparable to that observed in cases of hemochromatosis. The question, however, of whether the disease described in these cases is actually hemochromatosis has not yet been clarified.

Our interest in this subject was stimulated initially when an attempt was made to distinguish accurately between cases of hemochromatosis and of hemosiderosis on the basis of biopsy of the skin or liver. Inasmuch as the morphologic features of these two conditions may reveal similarities, it was considered worth while to conduct a parallel study of cases of hemochromatosis and transfusional hemosiderosis in which necropsy had been performed at the Mayo Clinic.

MATERIAL AND METHODS

This study included eighteen cases of hemochromatosis and sixteen cases of transfusional hemosiderosis in which necropsy had been performed some time during the years 1920 to 1951. In the latter cases diseases such as hemolytic anemia, leukemia and refractory anemia prevailed; all patients had received ten or more transfusions of blood, and pathologic examinations had revealed large deposits of iron in various organs. The clinical and laboratory data in the two groups of cases were studied and compared, but the main purpose of this investigation was comparison of morphologic features. It was hoped that such a comparison might afford some clues as to the role of excessive intake and storage of iron in the pathogenesis of hemochromatosis. Gross specimens from both groups of patients were studied. Histologic examination of sections of tissues was carried out although in some cases of hemochromatosis certain tissues were not available. For example, in only two cases were synovial membranes studied. Hematoxylin and eosin and Prussian blue stains were employed, the latter being used for identification of deposits of iron. Several specimens of tissues from both groups of cases were analyzed chemically to determine the amount of iron they contained.

HEMOCHROMATOSIS

There were seventeen men and one woman (Case 17) in this group. Their ages at the time of death ranged from thirty-two to eighty years, the average age being fifty-nine years. The age at clinical onset of the disease usually antedated the age at death by two to three years. The patients in Cases 1 and 18 had had symptoms of hemochromatosis for five years, in Case 11 for seven years and in Case 13 for twenty-five years.

Pathologic Aspects. A summary of the pertinent gross pathologic findings in each case of hemochromatosis is seen in Table 1. Cirrhosis of the liver was present in all eighteen cases. The weight of the livers varied from 750 to

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5,277 gm., the mean weight being 2,191 gm. Invariably, the liver was reddish brown, firm and hard; in all cases it was diffusely granular. (Fig. 1*a*.) The regenerative nodules were 0.5 cm. or less in diameter. The nodules were situated near one another and the atrophic zones were narrow. There were five cases of chronic perihepatitis. In one case a hepatoma involved both lobes of the liver. (Fig. 1*b*.)

The weight of the spleens ranged from 158 to 730 gm., and the mean weight was 348 gm. In most cases the spleen had increased in firmness. In fourteen cases chronic perisplenitis was present. The color of the spleen was not altered.

The smallest heart weighed 256 gm., the largest 825 gm., and the mean weight of the hearts was 396 gm. In most cases the heart was deep brown. Two patients had had chronic pericarditis and one had had generalized polyserositis. Pericardial effusion was present in four instances. Ascites had been present in fifteen of the seventeen cases in which this information was available. The ascitic fluid was invariably clear and yellow.

TABLE 1

SUMMARY OF PERTINENT GROSS PATHOLOGIC FINDINGS IN EIGHTEEN CASES OF HEMOCHROMATOSIS

Case	Weight (gm.)			Ascites (cc.)	Pleural Effusion (cc.)		Causes of death	
	Liver	Spleen	Heart		Right	Left	Immediate	Contributory
1	1,650	300	...	.....	300	300	Congestive heart failure	Chronic polyserositis; pancreatic lithiasis
2	1,800	445	255	3,000	0	0	Acute circulatory collapse	Bronchopneumonia
3	2,045	440	255	2,000	100	75	Generalized peritonitis	.....
4	1,800	370	510	300	1,000	1,000	Congestive heart failure	.....
5	2,920	585	350	200	50	0	Septicemia; erysipelas	Erysipelas, buttocks and scrotum
6	1,940	730	530	2,000	200	200	Myocardial failure	Acute hemorrhagic gastritis
7	2,650	370	400	800	0	0	Tuberculous peritonitis	Right iliofemoral thrombophlebitis
8	2,235	160	290	100	0	150	Septicemia; uremic coma	Pelvic and wound abscess; chronic rheumatic mitral endocarditis
9	2,360	230	550	0	500	100	Congestive heart failure	Chronic pericarditis
10	2,630	365	325	2,500	50	50	Uremic coma	Pulmonary edema; gastric ulcer; hepatoma; acute glomerulonephritis
11	1,485	395	825	2,000	1,800	2,000	Hepatic insufficiency	Cholelithiasis; chronic adhesive pericarditis
12	5,280	...	575	1,500	500	1,500	Pulmonary edema	Pulmonary embolus
13	4,800	545	395	2,000	0	0	Hepatic insufficiency	.....
14	750	440	345	20,000	0	0	Hepatic insufficiency	Cholelithiasis; pulmonary edema; bronchopneumonia
15	1,240	195	335	4,000	0	0	Hepatic insufficiency	Fracture neck right femur; hemorrhagic gastric ulcer
16	1,085	365	470	0	500	0	Bronchopneumonia	Cholelithiasis; metastatic squamous cell carcinoma tongue
17	2,070	250	410	2,000	200	100	Hepatic insufficiency	Thrombosis, portal and splenic veins
18	825	85	305	4,000	1,000	1,000	Congestive heart failure	Bronchopneumonia; atelectasis lower lungs; multiple esophageal and gastric ulcers

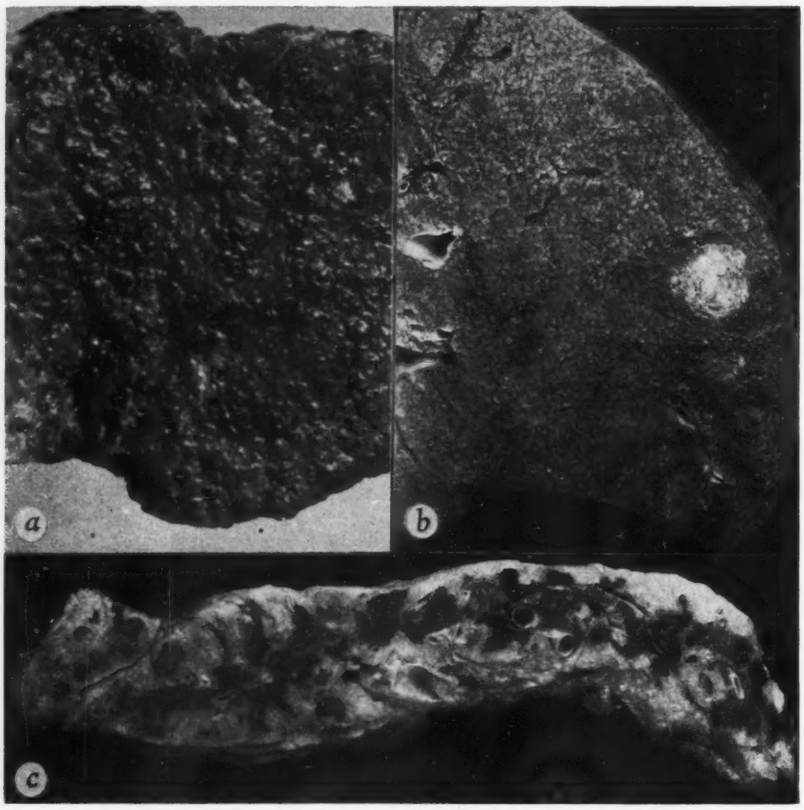

FIG. 1. *a*, Superior surface of right lobe of liver in case of hemochromatosis. Cirrhosis, granular type, is present; also small-sized, uniform regenerative nodules. *b*, Cut surface of left lobe of liver. Cirrhosis. Hepatoma. *c*, Cut surface of pancreas showing infiltration with fat and fibrous connective tissue in case of hemochromatosis.

The smallest kidney weighed 124 gm., the largest 378 gm. and the mean weight of the kidneys was 394 gm. Esophageal varices were demonstrated in five cases (Cases 10, 12, 14, 16, 17) and rupture had occurred in one of these cases (Case 17). Bilateral pleural effusion was noted in ten cases and unilateral pleural effusion in three, the smallest amount of fluid recorded being 50 cc., the largest 2,000 cc., and the average amount unilaterally 500 cc.

In twelve cases the pancreas was hard, firm and atrophic. (Fig. 1*c*.) In all but one case the pancreas varied from shades of brown to leaden gray. Testicular atrophy was disclosed in five cases. There was one case of tuberculous peritonitis.[1]

The parietal peritoneum, the stomach and the small and large intestines were pigmented externally in most cases. The lungs were normal in color and bronchopneumonia was present in four cases and pulmonary edema in three. In six cases the thyroid gland was deep brown. Grossly the adrenal glands usually appeared to be normal.

Histologic Appearance. The liver showed the characteristic features of Laennec's cirrhosis and abundant deposits of hemosiderin were present in the regenerative nodules and internodular stroma. (Fig. 2*a*.) The structural pattern was altered uniformly and the hepatic veins located eccentrically within or in the vicinity of the peripheral stroma. The regenerative nodules were closely set and usually 0.5 cm. in diameter or smaller. The bizarre cellular forms commonly associated with postnecrotic cirrhosis were not present. In ten cases a mild to moderate degree of parenchymatous fatty infiltration of the liver occurred. Alcoholic hyalin was observed in the hepatic cells in two cases only. Focal necrosis, usually mild in intensity and located centrilobularly, was present in fifteen cases. Various types of leukocytic infiltration in the regenerative nodules were not common. The internodular stroma appeared as narrow zones, containing an

abundance of collagen, as shown by van Giesen's stain, and of lymphocytes. The number of bile ducts was moderately increased. The extensive distortion of both hepatic and portal venules by the regenerative nodules was a further characteristic noted on microscopic examination. Inflammation of these veins was not common. Hemosiderin, stained with Prussian blue, was present in larger amounts in the internodular stroma than in the regenerative nodules in all eighteen cases. This pigment could usually be demonstrated in largest amounts in the connective tissue, hepatic cells and Kupffer cells, and in the order mentioned. It appeared to be evenly deposited in the centrilobular and perilobular parts of the regenerative nodules. In all but two cases (89 per cent) hemosiderin was observed in the epithelium of the bile ducts, and in these cases it was present in moderate or large amounts. (Fig. 2*b*.) It was observed in the endothelium of the hepatic or portal veins in three cases. Iron was present in the stroma and carcinomatous cells of a hepatoma. A metastatic squamous cell carcinoma, however, did not reveal deposits of hemosiderin.

Invariably an extensive amount of iron was present in the acini, ducts, islets and stroma of the pancreas. (Fig. 2*c*.) Of nine cases of hemochromatosis associated with diabetes mellitus, deposits of hemosiderin in the islets of Langerhans were large in two, moderate in three and small in four. In the nine cases in which diabetes mellitus was not present deposits of hemosiderin in the islets were large in three, moderate in three, minimal in one and absent in two. Interlobular and intralobular fibrous connective tissue and interlobular fat were characteristic histologic changes in the pancreas.

Hemosiderin usually was present in small or moderate amounts in the splenic pulp and in the reticular cells or macrophages. The trabeculae and connective tissue in the spleen commonly contained small to moderate amounts of this pigment. It was present in one instance in a thickened splenic capsule. It was conspicuously absent in the malpighian follicles. In addition to deposits of iron, fibrosis was severe in two cases, moderate in two cases and mild in ten cases. Chronic hyalin perisplenitis occurred in fifteen cases. Large deposits of hemosiderin usually were present in the portal, pancreatic, mesenteric, mediastinal and retroperitoneal lymph nodes. The lymph nodes elsewhere in the body contained less pigment if any. Usually the amount of connective tissue was increased, and extensive deposits of hemosiderin were demonstrated either in phagocytes or in free masses in the sinuses of lymph nodes. The follicles were conspicuously free of hemosiderin. Variable amounts of melanin were present, usually in the deep layers of the epidermis, in six of sixteen cases in which this information was given. In ten of these sixteen cases the sweat glands and their adjacent connective tissue contained hemosiderin and it was demonstrated in the basement membrane in eight cases.

In the kidney hemosiderin was demonstrated in the renal tubular epithelium in thirteen cases. A minimal amount occurred in the cells of the proximal convoluted tubules in three cases, and a small or moderate amount occurred in the

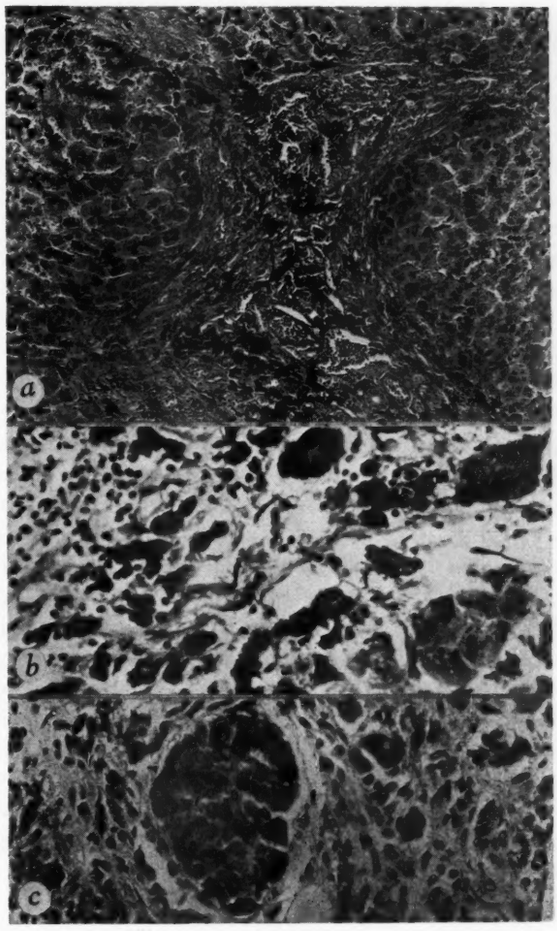

FIG. 2. *a*, Liver in case of hemochromatosis. Cirrhosis is present. Note deposits of hemosiderin in hepatic cells, Kupffer cells, stroma and bile ducts (Prussian blue stain). *b*, Same as in (*a*) (Prussian blue stain). *c*, Pancreas in case of hemochromatosis. Patient had diabetes mellitus. Hemosiderin in acini, ducts, stroma and islet (Prussian blue stain).

cells of the distal convoluted tubules and the loops of Henle in thirteen cases and in the cells of the collecting tubules in ten cases. In one instance the glomeruli contained a minimal deposit of hemosiderin in the endothelium. Chronic passive congestion was demonstrated in the kidneys in thirteen cases. So-called lower nephron nephrosis was demonstrated in Case 8, and severe acute glomerulonephritis was observed microscopically in Case 10.

The gastric glands in eight of twelve cases of hemochromatosis in which tissue was obtained showed deposits of hemosiderin, especially in the deep layer. Pigment was present in both the parietal and chief cells of the glandular epithelium of the stomach in two instances. The esophagus and the small and large intestines including the colon contained hemosiderin to much less degree than did the stomach. Sections of the duodenum were available for study in five cases and hemosiderin was present in two of them: in one case in Brunner's glands and in another case in the mucosa. It was present in the esophageal glands in one case, and in the mucosa of the gallbladder in one case. Microscopic examination of the lungs revealed variable degrees of chronic passive congestion, hemorrhagic edema and pneumonia. In four cases hemosiderin was demonstrated in the alveolar septa of the lungs.

Hemosiderin occurred in the muscle fibers of the heart in moderate or large amounts in thirteen of seventeen cases. The pigment was arranged at both longitudinal poles of the nucleus. Atrophy and focal fibrotic alterations in the myocardium were observed in six cases.

The histologic alterations of the adrenal glands in the seventeen cases of hemochromatosis in which sections were available were limited chiefly to the cortex. Hemosiderin was demonstrated in moderate to large amounts in the zona glomerulosa in eleven cases and in a minimal amount in four cases. A moderate amount was present in the zona fasciculata in three cases and a small amount in seven cases. In two cases deposition of hemosiderin in the adrenal gland was unusual in that a moderate amount occurred in the zona reticulosa, a mild amount in the zona fasciculata and none in the glomerular zona. The pigment was present in small amounts in the zona reticulosa in eight cases and in the medulla of the adrenal gland in one case.

In one of the two cases of hemochromatosis in which tissue from the synovial membrane of the knee joint was available, hemosiderin was deposited in the epithelial villi. It was present in the glandular epithelium of the prostate in seven cases and in the connective tissue of this organ in five cases. The germinal epithelium of the testes disclosed atrophy in five of fifteen cases. Hemosiderin was present in the seminiferous tubules in two cases and in the interstitial cells of the testes in eight instances.

The thyroid gland was frequently the site of histologic alterations. Usually an increased amount of fibrous connective tissue was found, and in nine cases adenomas were present. The epithelium of the gland was the commonest site of pigmentation and, in the fifteen cases in which sections were available, it was severe in three cases, moderate in three cases, mild in six cases and absent in three cases. Hemosiderin, although not observed in the colloid, was present in the connective tissue of the thyroid in three cases. The parathyroid glands, in cases of hemochromatosis, are typified by deep pigmentation. In four cases in which parathyroid tissue was examined microscopically, pigmentation was severe in the parenchymatous cells. In only two of eight cases was hemosiderin observed in minimal amounts in reticular cells in the bone marrow. Hemosiderin was not observed in the smooth muscle of the urinary and gastrointestinal tract; on the other hand, in two of five cases striated muscle contained minimal amounts. Tissue from the breasts of one man and one woman was available for study; in neither case did it contain deposits of hemosiderin. In one case the submaxillary gland contained a small amount of hemosiderin.

Clinical Aspects. There were seventeen males and one female. The youngest patient was thirty-two years of age and the oldest eighty years at the time of death. The mean age was fifty-nine years. In ten cases a correct diagnosis of hemochromatosis had been made on the basis of clinical factors. The diagnosis in five patients (Cases 8, 14, 15, 16, 18) could be considered subclinical in that the triad of diabetes, pigmentation and an enlarged liver was not fully present. The initial symptoms and the number of cases in which they were given were as follows: weakness unrelated to diabetes (seven cases); diabetes mellitus (two cases); pigmentation of the skin (two cases); ascites, ascites and jaundice, abdominal pain, enlarged liver, and dyspnea (one case each). In the nine cases in

which diabetes mellitus occurred it had been present for a year and a half in two cases, one year in three cases, a half year in three cases and one month in one case. Abdominal pain had been a symptom of nine patients. Thirteen patients had had ascites: one patient, an alcoholic, had had bouts of ascites for nine years; one had had ascites for one year, one for three months, three for two months, three for one month, two for one week; for two patients the period was unknown. Of the seven patients who had been jaundiced, one patient (Case 11), an alcoholic, had had recurrences of jaundice for nine years. Two patients had had jaundice for six months, two for one month, one for two weeks and one for one week.

Among the other symptoms noted were peripheral neuritis (Cases 5, 13), significant loss of hair (Cases 12, 13, 17), indigestion (Cases 5, 10, 13, 14, 15) and diarrhea (Cases 1, 13). Six patients (Cases 1, 5, 9, 11, 13, 14) had histories of alcoholism. The number of years of excessive alcoholic intake were as follows: Case 1, twenty years; Case 11, twenty-five years; Case 5, five or more years; Case 9, unknown; Case 13, twenty-five years; and Case 14, four years. All patients except three (Cases 8, 15, 16) had had marked weakness, and all except five (Cases 8, 13, 14, 15, 16) had had dyspnea.

In thirteen cases palpation had revealed that the patients' livers were enlarged. The five patients who had not had hepatomegaly were among the seven patients who had not had a clinical diagnosis of hemochromatosis. On palpation the spleens of four patients had been found to be enlarged. Surgical resection for an enlarged spleen had been carried out in Case 12. An enlarged heart was a physical finding in six patients. Fourteen patients had had edema of the ankles or legs. Spider angiomas were present in three cases, and palmar erythema was demonstrated in two cases. One patient (Case 14) had had a caput medusae. Eleven patients had lost from 10 to 40 pounds (4.5 to 18.1 kg.), the average loss being about 20 pounds (9.1 kg.). Three patients (Cases 10, 15, 17) had had massive gastrointestinal bleeding and one had had bleeding from a rectal carcinoma (Case 8). In three cases testicular atrophy was present. Thirteen patients had had pigmentation of the skin of variable character and distribution. The skin of one patient had been pigmented for six years, of another for three years, and of five patients for one year. Six patients had been unable to relate the duration of their pigmentation. Arterial hypertension, retinopathy or gynecomastia had not been observed. The commonest causes of death in these cases of hemochromatosis were hepatic insufficiency in five cases and congestive heart failure in four cases. In Case 12 blood had been transfused on several occasions after splenectomy, but pathologic examination of the spleen at the time of the operation had demonstrated extensive deposits of hemosiderin. In Case 17 a diagnosis of pernicious anemia of twenty-five years' duration had been made. Various hematonics had been employed at irregular intervals during that time. In Case 14 thromboangiitis obliterans had been diagnosed and whiskey had been taken on prescription for four years before cirrhotic features were noted clinically.

Laboratory Data. The average value for hemoglobin was 12.8 gm. per 100 cc. of blood; the highest value was 17.2 gm. and the lowest 4.7 gm. The numbers of erythrocytes varied from 2,720,000 to 5,040,000 per cu. mm. of blood, the average number being 3,860,000. In three cases, including a case with Banti's syndrome, the patients had had anemia, which is assumed to be uncommon in association with hemochromatosis. Leukocytosis was present in six cases and leukopenia in two cases. Blood smears disclosed three cases of macrocytosis. The value for direct serum bilirubin was elevated in six cases (1.2 to 11.3 mg. per 100 cc.) and for the indirect in five cases (1.6 to 7.2 mg. per 100 cc.). Sulfobromophthalein tests were made in seven cases and in six significant retention of dye was demonstrated as follows: retention, grade 1, one case; grade 2, three cases; grade 3, two cases. The value for serum protein was normal in all cases, but hyperglobulinemia (3.9 to 5.5 gm. per 100 cc.) was present in four of five cases in which determinations for serum protein were made. An increased value for urea (43 to 178 mg. per 100 cc. of blood) had been noted among twelve of fifteen patients. Ten of thirteen patients had had hyperglycemia (131 to 476 mg. per 100 cc.). Five patients had had albuminuria and two patients had had erythrocyturia of significant degree. Examination of the urine in Case 10 had revealed moderate amounts of albumin, erythrocytes and casts. Results of all biopsies of the skin of six patients were positive for hemosiderin. Needle biopsy of the liver had been performed in two cases and histologic examination had disclosed the presence of hemo-

chromatosis. Electrocardiograms in five cases had demonstrated the commonest abnormalities to be left ventricular strain pattern, inversion of all T waves, and slurred or low amplitude of all QRS components. In one case an auricular flutter was demonstrated and in another case auricular fibrillation was present. Further laboratory studies disclosed abnormal prothrombin time in four cases, abnormal values for cephalin-cholesterol flocculation in two, hypolipemia in one, low values for serum cholesterol-cholesterol esters in one, reduced number of blood platelets in one; urinary estrogens were absent in one case. Gastric analyses disclosed hyperchlorhydria in four cases and histamine achlorhydria in one (Case 17). Results of Kline and Kahn serologic tests were positive in Case 17.

TRANSFUSIONAL HEMOSIDEROSIS

The sixteen patients who had transfusional hemosiderosis were evenly divided between the two sexes.

Pathologic Aspects. Except for a generalized dark pigmentation of the skin and brownish coloration in the organs of one patient, gross pathologic characteristics which could be related to deposits of iron were not significant.

Histologic Appearance. Microscopically, the liver in most cases characteristically revealed abundant deposits of hemosiderin and absence of cirrhosis. In four cases moderate fatty infiltration of the liver had occurred. Centrilobular hepatic necrosis of mild degree in seven cases and of moderate degree in two cases had taken place. Cirrhosis of the liver, present in only one case, was associated with necrosis of hepatic cells and fatty change. In this case hemosiderin was absent in the portal spaces and epithelium of the bile ducts and present in minimal amounts in the hepatic cells and Kupffer's cells. Pigmentation in the hepatic cells was severe in five cases, moderate in two cases and minimal in nine cases. Large deposits of hemosiderin were demonstrated in Kupffer's cells in five cases, moderate amounts in seven cases and minimal amounts in four cases. In most cases the hemosiderin was located in the perilobular area of the hepatic parenchyma. It was present in small to moderate amounts in eleven cases in the connective tissue of the portal triads. In five cases (31 per cent) deposits were demonstrated in the epithelium of the bile ducts. (Fig. 3*a*.) Four patients, two of whom had had idiopathic hemolytic anemia, had maximal deposits of hemosiderin in the hepatic parenchyma and portal triads. Hemosiderin was present in the endothelium of the hepatic veins in one case. A metastatic retroperitoneal embryoma of the kidney was present in the liver in one man, but the tumor itself did not contain deposits of hemosiderin.

Hemosiderosis of the pancreas occurred in three cases. Two patients had had large deposits of hemosiderin in the acini, ducts, islets and stroma of the pancreas (Fig. 3*b*) and one patient (Case 11) had had minimal deposits in the acini and ducts. Minimal fibrosis of the pancreas was present in eight cases, and fatty infiltration was demonstrated in mild degree in five cases.

Deposits of iron were observed in the spleen in all but one case. Hemosiderin was present in macrophages or extracellularly in the pulp in large amounts in five cases, moderate amounts in four cases and minimal amounts in six cases. It was conspicuously absent in the malpighian follicles of the spleen, and was present in small to moderate amounts in the stroma in eight cases. In eight cases also the spleen was the site of mild to moderate fibrosis. Chronic perisplenitis was present in two cases. A hemorrhagic infarct of the spleen and thromboses of the splenic veins were further histologic observations. Various lymph nodes were examined for deposits of hemosiderin and large amounts were present in two cases, moderate amounts in two and small amounts in two. Whereas in the cases of hemochromatosis the lymph nodes, especially those located in the abdomen, had a characteristic abundance of hemosiderin, in the cases of transfusional hemosiderosis they had a smaller amount which did not seem to vary with the location of the node. Melanin was present in the basal layer of the epidermis in two cases. Hemosiderin was present in the sweat glands and derma in two of six cases in which sections were available for study.

Deposits of hemosiderin occurred in the renal tubular epithelium in eight cases. Only small deposits were present in the proximal convoluted tubules in five cases. In the distal convoluted tubules and loops of Henle in four cases deposits were minimal and in four cases they were moderate. In the collecting tubules deposits were small in four cases and moderate in three. In fifteen of the sixteen cases there was mild to moderate chronic passive congestion of the kidneys. Lower nephron nephrosis was pres-

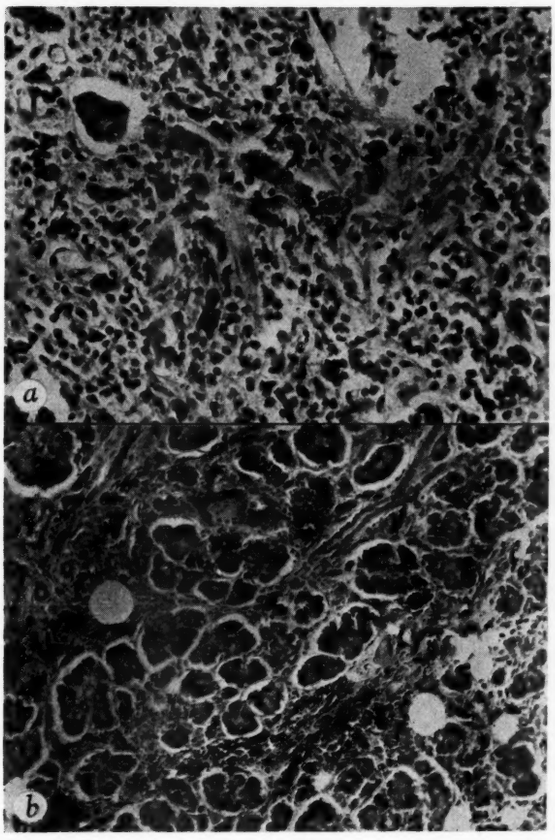

FIG. 3. Transfusional hemosiderosis. *a*, Liver; deposits of hemosiderin in hepatic cells, Kupffer cells, portal area and bile ducts (Prussian blue stain). *b*, Pancreas shows hemosiderin deposited in acini, ducts and islet (Prussian blue stain). Patient did not have diabetes mellitus.

ent in one case, and hemorrhages had occurred and hemoglobin casts were present in the renal tubules in one case. Hemosiderin was absent in sections of the esophagus, stomach and small and large intestine including the rectum with one exception. This patient had large deposits in all portions of the gastric glands (Fig. 4*a*) and in the septa of the lungs. Three other patients had had small deposits in the septa of the lungs.

The lungs were commonly the site of chronic passive congestion and hemorrhagic edema. Pneumonia and atelectasis were present in two cases and pulmonary emboli in one case. A moderate amount of hemosiderin occurred in the myocardium in one case only. The pigment was arranged longitudinally and at the bipolar ends of the nucleus of the muscle fibers. (Fig. 4*b*.) In two cases the adrenal cortex was the site of deposits of hemosiderin. (Fig. 4*c*.) Deposits of hemosiderin were not demonstrated in the prostate glands. Minimal amounts of hemo-

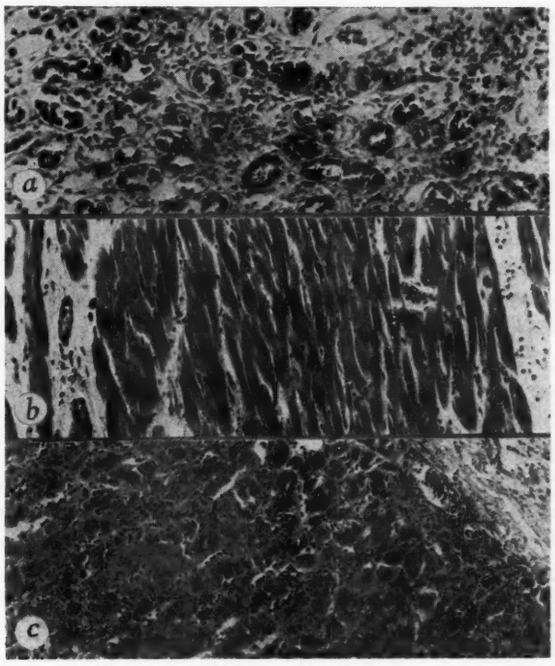

FIG. 4. Transfusional hemosiderosis. *a*, Stomach; hemosiderin is deposited in the gastric glands (Prussian blue stain). *b*, Heart showing hemosiderin in myocardial fibers (Prussian blue stain). *c*, Deposits of hemosiderin are shown in the zona glomerulosa and zona fasciculata of the adrenal gland (Prussian blue stain).

siderin were present in the seminiferous tubules and interstitial tissue of the testes of one patient. Hemosiderin was present in the thyroid gland in three cases. The parathyroid glands in two cases were available for study, but extensive deposits of hemosiderin in both types of cells were found in only one. Histologically, minimal deposits of hemosiderin were revealed in the bone marrow in four cases and maximal amounts in two cases. In the bone marrow hemosiderin was present either intracellularly or in macrophages. It was present in striated muscles in small amounts in one of six cases.

Clinical Aspects. There were eight males and eight females and the ages varied from one year to seventy-eight years. The mean age of the adults was forty-nine years. Three of the patients were children, seven, five and one year of age, respectively. The duration of illness in these cases varied from three months to nine years. The clinical diagnoses in these cases were as follows: acute lymphatic leukemia in three cases, chronic lymphatic leukemia in two cases, hemolytic anemia in three cases and chronic ulcerative colitis, Wilms's tumor, Laennec's cirrhosis, plasma cell myeloma, reticulum cell sarcoma,

thrombocytopenic purpura, bleeding duodenal ulcer and pancytopenia in one case each. All of these diagnoses were confirmed at necropsy. Multiple transfusions of blood were administered to all patients except one either for persistent or for refractory anemia. The number of transfusions of 500 cc. each given to these patients varied from 10 to 291. Usually the transfusions were administered during a period ranging from several months to three years. Two exceptions were noted: one patient who had had chronic ulcerative colitis for nine years and had undergone colectomy had required thirty-nine transfusions of blood during the last four years of his illness; another patient had Laennec's cirrhosis and had required transfusions of blood over a six-year period because of recurrent hemorrhage from esophageal varices and persistent anemia and during an operation for a portacaval shunt.

One case is of unusual interest. The patient had had chronic lymphatic leukemia for three years and because of refractory anemia had required at least 291 transfusions of blood. He had experienced several reactions attributable to the transfusions during this period. The condition of three patients was diagnosed as idiopathic hemolytic anemia and multiple transfusions of blood were given in addition to corticotropin. It is recognized that in these cases the deposition of some of the hemosiderin may have been the result of hemolysis which occurred prior to the administration of blood. In other words, some hemosiderosis occurred as a complication of the patients' disease prior to the transfusions.

Six patients had undergone operations not previously mentioned as follows: nephrectomy, one case; splenectomy, four cases; gastric resection, one case.

In the eleven cases in which reports on the marrow were available it was found to be normal in two cases. Myeloma occurred in one case; hyperplasia in three cases; hyperplasia associated with myeloid and lymphoid elements in one case; acute lymphatic leukemia in two cases and hyperplasia in two cases.

DETERMINATION OF IRON IN TISSUES

In both groups of cases the amount of iron contained in various organs at necropsy was determined chemically. (Table II.)* According to Muirhead[2] the normal value for iron in the liver is 16.6 mg. and in the pancreas, 7.37 mg., each in 100 gm. of wet tissue. Sheldon[3] stated that the average lymph node in a case of hemochromatosis contained 7.92 per cent iron by dry weight. In livers from four patients in our group of cases of hemochromatosis the total amounts of iron were 27.15, 26.48, 10.82 and 20.16 gm., respectively, or about twenty-five to sixty times the normal values of iron in the liver. Sheldon considered 21.36 gm. as the average total amount of iron in the liver of a patient having hemochromatosis. In two cases of hemochromatosis 340 and 168 mg. of iron were present in the pancreas, diabetes mellitus being present in both cases. That the pancreases in these two cases of hemochromatosis contained from twenty-two to forty-five times the normal amount of iron is apparent. One abdominal lymph node in a case of hemochromatosis was assayed and found to contain 2,043 mg. of iron per 100 gm. of tissue.

TABLE II
QUANTITATIVE DETERMINATIONS FOR IRON

Case	Organ	Weight (gm.)	Milligrams of Iron per 100 gm. Wet Tissue	Total Iron in Organ (gm.)
Hemochromatosis:				
5	Liver	2,920	930	27.15
7	Liver	2,648	1,000	26.50
	Pancreas	.....	340	.....
11	Liver	1,485	740	10.82
13	Liver	4,800	420	20.16
	Pancreas	.....	168	.....
	Lymph node	.....	2,043	.....
Transfusional hemosiderosis:				
19	Liver	1,950	101.5	1.98
21	Liver	650	8.1	0.05
	Pancreas	100 (estimated)	6.7	0.007
25	Liver	4,550	416	18.90
	Pancreas	125	412	0.52

In three cases of transfusional hemosiderosis the quantities of iron in the tissues were abnormally high. The entire liver of one patient contained about six times the normal amount of iron and that of another, about twenty-five times the normal content. The pancreas of the

* We are indebted to Dr. Marschelle H. Powers, Section of Biochemistry, Mayo Clinic, for the analyses of iron in tissues.

latter of these two patients contained about fifty-five times the normal amount of iron.

COMMENT

From the results of this study it is apparent that hemochromatosis and transfusional hemosiderosis are two separate and distinct entities. A comparison of eighteen cases of hemochromatosis and sixteen cases of transfusional hemosiderosis showed definite differences, the most important being indicated in Table III. In general, striking clinical similarities were not noted. Histologically it was noted that in cases of transfusional hemosiderosis iron in the hepatic cells was perilobular in distribution and it was present in the epithelium of the bile ducts in only a few cases. However, the amount and distribution of hemosiderin in the spleen, kidneys, lungs, parathyroid glands and bone marrow was similar in both groups of cases. Hemosiderin was not identified in the smooth muscle of the urinary and gastrointestinal tracts in either group of patients.

TABLE III
NOTABLE DIFFERENCES BETWEEN EIGHTEEN CASES OF HEMOCHROMATOSIS AND SIXTEEN CASES OF TRANSFUSIONAL HEMOSIDEROSIS

Difference	Hemochromatosis (Cases)	Transfusional Hemosiderosis (Cases)
Sex: male	17	8
female	1	8
Cirrhosis of liver	18	1 (Case 21)
Diabetes mellitus	9	0
Pigmentation of skin	13	1
Enlarged liver	13	1
Atrophy of pancreas	12	0
Testicular atrophy	5 (15*)	0
Deposits of hemosiderin in:		
Pancreas	18	3
Gastric glands (deep areas)	8 (12)	1
Myocardium	13 (17)	1
Adrenal gland:		
Zona glomerulosa	15 (17)	2 (12)
Zona fasciculata	10 (17)	1 (12)
Zona reticulosa	8 (17)	1 (12)
Thyroid gland, epithelium	12 (15)	2 (10)
Sweat glands and derma of skin	10 (16)	2 (6)
Prostate gland:		
Epithelium	7	0
Connective tissue	5	0
Testes:		
Seminiferous tubules	2	1
Interstitial tissue	8	1

* Numbers in parentheses indicate number of cases in which information was available when entire series of cases was not considered.

In the interpretation of the differences and similarities of these two diseases a review of the case of the patient who received 291 transfusions of blood will be helpful. The content of iron in various tissues as determined chemically was equivalent to or even greater than the amounts found in true cases of hemochromatosis but neither cirrhosis of the liver nor diabetes mellitus was present. It can, of course, be argued that if the patients who had transfusional hemosiderosis had lived as long with their overload of iron as patients who had hemochromatosis, the clinical and pathologic characteristics of hemochromatosis would have then become evident. In this connection it is interesting to note that in five cases of hemochromatosis (Cases 8, 14, 15, 16, and 18) the disease had not reached a stage of development when a definitive clinical diagnosis could be made. These patients died of causes unrelated to the disease. Nevertheless, all of these cases were significantly distinct from the cases of transfusional hemosiderosis in that the patients had cirrhosis of the liver, and hemosiderin was present in greater amounts in the tissues in general, and specifically in the epithelium of the bile ducts, the portal triad of the liver, the endocrine glands, the myocardium and the pancreas.

It is admitted that the amount of iron observed histologically in some cases of transfusional hemosiderosis appeared out of all proportion to the amount administered by transfusions of blood. Furthermore, it is recognized that in cases of hemolytic anemia, for the most part, and possibly in cases of leukemia and pancytopenia, deposits of hemosiderin in various organs may occur in the absence of transfusions. During multiple transfusions of blood reactions may occur causing hemolysis of erythrocytes and consequent deposition of hemosiderin in the body.

Critical study of our cases and those reported in the literature indicates that hemochromatosis is a unique disease and that simply overloading the body with iron has not reproduced conclusive or convincing evidence of the disease in either human beings or experimental animals. In this concept our study supports the contentions of Dry,[4] Althausen and Kerr,[5,6] Sheldon,[3]

Muir and Young,[7] Wyatt, Mighton, and Moragues,[8] Butt and Wilder,[9] and others.[10] We have concluded also that some factor or factors other than malnutrition and overloading with iron account for the development of hemochromatosis. That the disease represents an inborn error of metabolism beginning in infancy has been the opinion of many of these investigators. Granick[11,12] has demonstrated normal amounts of ferritin in the duodenal mucosa and in the liver in cases of hemochromatosis, supporting the contention that at least a normal intestinal mechanism exists for the absorption of iron although it may be working to excess.

A number of workers have reported the development of hemochromatosis among patients having various types of anemia,[13–15] iron overload from multiple transfusions of blood,[16–20] malnutrition[21,22] and avitaminosis.[23] Critical survey of these reports indicates that in many cases the so-called hemochromatosis does not fulfill the criteria necessary for a diagnosis of true hemochromatosis. To be specific, many patients do not have pigmentation of the skin,[17] diabetes mellitus,[14,17,21,22] or cirrhosis of the liver,[13,16,17,21–23] and hemosiderin in various tissues is not distributed typically or in the quantities encountered in cases of hemochromatosis.[16,17,21,22]

Cirrhosis of the liver is characterized morphologically by the presence of regenerative nodules, increased amounts of internodular fibrous connective tissue and necrosis of the hepatic parenchyma. Some authors use the term "cirrhosis of the liver" for any lesion in which the amount of fibrous connective tissue in the liver is increased. That this latter concept of cirrhosis of the liver is erroneous is apparent. In many cases a diagnosis of hemochromatosis has been made when true cirrhosis of the liver has not been present. Because of these differences it is our opinion that such cases should be designated by the broad term "hemosiderosis" with an appropriate adjective indicating the origin of the iron overload, and that the term "hemochromatosis" should be reserved for the definitive disease as described in this study.[2,8,19,24–28] In addition, gross and histologic examinations of tissue in cases of transfusional hemosiderosis demonstrate minimal evidence of necrosis of tissue due to deposits of hemosiderin. Even after 291 transfusions of blood in one case significant damage to the tissues was not revealed. That significant organic damage owing to iron overload occurs in cases of transfusional hemosiderosis is to be questioned.

Many investigators have attempted to reproduce hemochromatosis experimentally by means of various methods. Taylor, Stiven, and Reid[29] have produced an idiopathic hemosiderosis in cats on the basis of malnutrition and lack of sufficient vitamin A in the diet. In our cases of hemochromatosis and transfusional hemosiderosis we were unable to discover any nutritional, mineral or vitamin deficiency with the possibility of one exception, Case 14. This was a case of thromboangiitis obliterans in which part of the treatment consisted of consumption of alcohol. Several years later cirrhosis of the liver was diagnosed clinically, and subclinical hemochromatosis was demonstrated at necropsy. Kinney, Hegsted and Finch[30] showed that in rats increased amounts of iron were absorbed and deposited in the liver and other organs after the administration of a diet deficient in proteins, minerals and vitamins and the supplementary addition of ferric citrate. These investigators further demonstrated increased absorption and storage of iron in rats fed a diet deficient in phosphate. Finch and co-workers[31] found deposits of iron distributed in various tissues after excessive absorption of iron or parenteral administration of iron in experimental animals and believed they had reproduced the clinical and pathologic picture of hemochromatosis. However, they do not give convincing evidence that cirrhosis of the liver, which is a basic condition relative to hemochromatosis, was produced experimentally. After critical survey of their excellent work we are of the opinion that, histologically, their results simulate those in our cases of transfusional hemosiderosis rather than of hemochromatosis. The comparison of results obtained in experimental animals with those obtained in patients, however, is known to be difficult. Most investigators employing different compounds of iron or intravenous administrations of blood have failed to produce hemochromatosis in experimental animals.[32–38]

This study indicates that both clinical and pathologic evidence is necessary for a diagnosis of hemochromatosis and to differentiate it from hemosiderosis. Factors and tests which may be helpful clinically in making the differential diagnosis are: the presence of iron in the propria of the sweat glands and the upper part of the cutis of the skin;[39] the presence of hemosiderin in the gastric specimens at biopsy;[40] needle

biopsy of the liver;[41–43] high concentration of iron in serum;[44] intravenous iron tolerance test;[45,46] intracutaneous ferric chloride test,[47] and the identification of hemosiderin in the urine. That laboratory tests may reveal minimal evidence of hepatic disease in the presence of extensive histologic changes in the liver in cases of hemochromatosis has been recognized. Commonly evidence of retention of sulfobromophthalein dye has not been noted after one hour in cases of hemochromatosis. Above all it is to be stressed that needle biopsy of the liver is the most reliable method of diagnosis of hemochromatosis. If finely granular cirrhosis is observed in association with extensive deposits of hemosiderin, the diagnosis of hemochromatosis is practically confirmed.

SUMMARY AND CONCLUSIONS

As a result of our studies of eighteen cases of hemochromatosis and sixteen cases of transfusional hemosiderosis based on observations at necropsy at the Mayo Clinic, we have concluded that the two conditions are separate and distinct entities. Hemochromatosis may be defined clinically as a slowly progressive disease which occurs almost exclusively in the male. Diagnosis is usually made during the fifth or sixth decade of life. Cirrhosis of the liver is always present and diabetes mellitus, pigmentation (melanosis) of the skin and asthenia often occur. In the cases that we have studied, iron overload, attributable to transfusions of blood or to any other cause has not lead to hemochromatosis.

Transfusional hemosiderosis occurs with equal frequency among the two sexes and 25 per cent of our patients were twenty-five years of age or less. Cirrhosis of the liver is absent or a coincidental finding and diabetes mellitus and pigmentation of the skin occur infrequently.

Pathologically, in cases of hemochromatosis a finely granular cirrhosis is invariably present and usually associated with fibrosis of the pancreas, atrophy of the testes and pigmentation of the viscera. Histologically, extensive deposits of intracellular hemosiderin occur in the pancreas, lymph nodes, endocrine glands, heart and stomach in hemochromatosis, but are unusual in transfusional hemosiderosis. Deposits of iron in the bile ducts and parenchymatous damage to the pancreas are usually demonstrable in hemochromatosis but are infrequent in hemosiderosis.

Needle biopsy of the liver is the most reliable method of diagnosis of hemochromatosis. Death is most commonly due to hepatic insufficiency or myocardial hemosiderosis.

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The Electrocardiogram in Potassium Depletion*

Its Relation to the Total Potassium Deficit and the Serum Concentration

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DESPITE the current widespread interest in the electrocardiogram of potassium depletion, it has not yet been clearly established whether the electrocardiogram is a reliable clinical guide to this condition. Nor has it been determined whether the electrocardiogram is most closely related to the level of serum potassium, the tissue deficit of potassium or to some ratio between these two. The purpose of the present report is to analyze the correlation between these parameters in a group of subjects showing varying degrees of spontaneous or experimental potassium depletion.

METHODS

Twelve electrolyte and nitrogen balance studies were carried out on nine normal subjects in whom potassium depletion was produced by the administration of desoxycorticosterone acetate (DOCA®), 17-hydroxycorticosterone acetate (compound F),† ammonium chloride or ammonium sulfate. Additional balance studies were made on two patients with severe chronic potassium depletion induced by overuse of laxatives. The detailed balance data from most of these studies, as well as the chemical methods employed, are reported elsewhere.[1–4] None of the subjects had any clinical evidences of cardiovascular disease and none were receiving digitalis or other cardiac drugs. In each of these studies frequent determinations were made of serum potassium, sodium, chloride, carbon dioxide content and pH. Serum calcium concentrations were measured occasionally in most subjects. Changes in the balance of potassium and the other electrolytes were calculated from the analysis of diets, stools and urine. The cumulative potassium balance was calculated in two ways: (1) without reference to the nitrogen balance ("K_{Bal}") and (2) after correction for the cumulative change in nitrogen balance ("K_N"). This latter calculation is assumed to correct for changes in potassium balance due to fluctuations in protoplasmic mass.[5] Throughout this paper individual values for K_{Bal} and K_N are expressed as cumulative loss or retention, using as a baseline or "zero balance" the total body potassium on the last control day before any experimental procedure was instituted. In the two patients recovering from diarrhea (R. S. and A. B.), the final total retentions of potassium were considered to restore total body potassium to normal ("zero balance"), and therefore the preceding values for balance were calculated back from the final day of study. The validity of this assumption was supported by serial K^{42} measurements.[2]

In the majority of instances electrocardiograms were recorded at the start of each day but in a few cases they were taken less frequently. Nine or twelve leads (6 limb leads and V_2, V_4 and V_6 or V_1–V_6) were recorded with a direct-writing amplifier-type electrocardiograph. For purposes of classification and analysis no serum change smaller than 0.5 mEq./L. and no balance change smaller than 40 mEq. was considered significant.

The electrocardiograms were read independently by one of us (H. D. L.) without knowledge of the chemical data, and were arbitrarily classified according to the following scale:

† Compound F was supplied through the courtesy of Dr. Elmer Alpert, Medical Division, Merck and Co., Rahway, New Jersey.

* From the Medical Services of the New England Center Hospital, the Peter Bent Brigham Hospital and the Evans Memorial, Massachusetts Memorial Hospitals; and the Departments of Medicine, Tufts College Medical School, Harvard Medical School and Boston University School of Medicine, Boston, Mass. Supported in part by grants from the Institute for Arthritis and Metabolic Diseases and the National Heart Institute of the National Institutes of Health, United States Public Health Service, and from the Greater Boston Chapter, Massachusetts Heart Association.

+1 = Normal complexes with symmetrical peaked ("tent-shaped") T-waves.
0 = Normal or within normal limits.
−1 = Lowering of the T-wave with relative or absolute prominence of the U-wave, the T-wave still exceeding the U-wave in height.
−2 = Further lowering of the T-wave with increase in the height and/or duration of the U-wave, the T and U-waves being equal in height.
−3 = Still further lowering of the T-wave, which is now flat or inverted, and further increase in the height and/or duration of the U-wave, so that the U-wave is taller than the T-wave.

This classification, illustrated in Figure 1, attempts to quantitate the apparent degree of potassium depletion indicated by the electrocardiogram. The changes are seen mainly in lead V_2 in male subjects and in V_4 in females. The validity of these classifying criteria is supported by the clinical experience of one of us (H. D. L.), but if the more conventional criteria of RS-T segment depression or apparent prolongation of Q-T interval[6,7] are employed, the classification of electrocardiograms is not materially altered. In this paper an algebraic increase (change in the plus direction) in the classification number is said to represent an "ascending" change, and an algebraic decrease (change in the minus direction) is called a "descending" change in the electrocardiogram. In addition to the schema outlined above, a sixth category was used for the classification of abnormal tracings which did not suggest potassium depletion but did show low, flat or inverted T-waves without change in the T-U contour. Such non-specific tracings were called abnormal ("Abn"). No variation in the electrocardiogram was considered significant unless it resulted in a change of classification as outlined above.

Using these criteria, thirty-three sequences in which there were significant changes in serum potassium, potassium balance or the electrocardiogram were selected from the total material. Analysis of these data forms the subject of this report.

RESULTS

Effects of Acute Changes in Serum Potassium and Tissue Balance on the Electrocardiogram. In Table 1 are summarized thirty-one instances in which there was a significant change in serum potassium or tissue balance or in both. Also included are two instances of change in the electrocardiogram without change in either serum concentration or potassium balance. The data include the clinical or experimental circumstances producing the observed change, the duration of each observation, the values for serum potassium, K_{Bal}, and K_N at the beginning and end of the period of observation and, in the patients receiving acidifying salts, the serum bicarbonate concentrations. These are compared with the simultaneous changes in the electrocardiographic classification.

The first three sections of Table 1 (A, B and C) are concerned with patients who, with two exceptions (R. S. and W. W., section B), were developing potassium depletion. In most instances (section A) there were significant losses of potassium before definite reduction in serum concentration developed.

Section A: In this group there were eleven cases with a loss in K_{Bal} of from 42 to 175 mEq. over periods of one to fourteen days. In six of these cases there was no significant change in the electrocardiogram. In four cases the electrocardiogram showed "descending" changes, and in the eleventh case the tracings showed "ascending" changes.

The absence of correlation between the magnitude of the change in K_{Bal} and the development of "descending" electrocardiographic abnormality is emphasized by the fact that the four cases of this group who exhibited these changes suffered relatively small losses in K_{Bal}.

In two cases in this section (G. S. and W. W.) K_N actually increased although K_{Bal} decreased. In one of these cases the electrocardiogram seemed best correlated with the change in K_N and in the other, with K_{Bal}. However, for reasons outlined below, these patients are classified according to the change in K_{Bal}.

There were no significant changes in other serum electrolytes in eight cases but in the other three (H. K., J. M. and F. W.) serum pH and CO_2 was moderately reduced by the administration of acid-forming salts. None of these latter subjects showed electrocardiographic changes.

Section B: Section B of Table 1 includes four instances in which the serum potassium was significantly decreased while the balance was unchanged. Despite the fact that the reduction

in serum potassium was essentially the same in all patients, two of this group showed "ascending" and two showed "descending" electrocardiographic changes.

Section C: There was only one sequence chosen for study in which serum potassium and potassium balance decreased simultaneously. In this case, despite a cumulative loss of 101 mEq. over seven days and a drop in serum concentration of 0.7 mEq./L., there was no definite change in the electrocardiogram.

Sections D and E: Sections D and E describe fifteen sequences during recovery from potassium depletion. In eight of these sequences (section D) there was a simultaneous increase in serum concentration and in balance. In five of these latter cases, despite retentions of from 104 to 350 mEq. of potassium over two to six days and increments in serum concentration of from 0.5 mEq./L. to 1.7 mEq./L., there was no significant change in the electrocardiogram. In the other three cases who during three to five days retained 103 to 144 mEq. and had a rise of 0.6 mEq./L. in serum concentration, the electrocardiogram showed "ascending" changes. Here again, there was no correlation between the magnitude of acute changes in balance and serum concentration and the effect on the electrocardiogram.

Section E: Section E describes seven instances in which the serum did not change significantly despite increments of 48 to 196 mEq. in K_{Bal}. Without consistent relation to the magnitude of the balance changes, the electrocardiogram showed "ascending" changes in three instances, and did not change in the other four.

Section F: This section describes two patients whose electrocardiograms showed "descending" changes despite the absence of significant alterations in serum concentration or K_{Bal}. In one case (J. McL.), the electrocardiographic change may have reflected a delayed response to an acute loss of 44 mEq. in K_{Bal} which had occurred on the first day of treatment with compound F (section A) just prior to the period under consideration. The other patient, G. S., showed a definite "descending" change in the electrocardiogram during a period of control observation.

Relationship between the Magnitude of the Potassium Deficit and the Electrocardiogram. In Figure 2 are plotted forty-nine simultaneous potassium balance and electrocardiographic observations taken from the data in Table I. Each point represents a single observation in one patient, plotted on two coordinates. The abscissa is the potassium balance, positive or negative, calculated for the morning on which the electrocardiogram was recorded. The ordinate is the classification number of the electrocardiogram (+1 to −3). Each point is drawn in one of three ways according to the serum potassium level: a solid triangle for concentrations below 3.0 mEq./L.; a solid oval for concentrations between 3.0 and 3.7 mEq./L.:

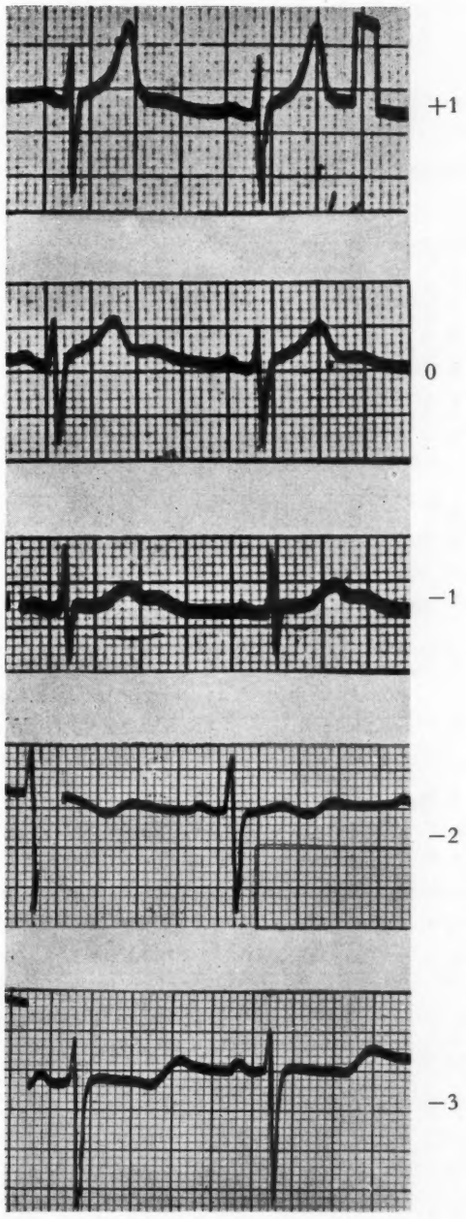

FIG. 1. Illustrative examples of the electrocardiographic classification used in this study. All tracings were recorded from lead V_2.

TABLE 1

EXPERIMENTAL DATA

A. Sequences in Which Serum Potassium Was Unchanged and Potassium Balance Was Negative

Patient	Procedure	Duration (days)	K_S (mEq./L.)		K_{Bal} (mEq.)		K_N (mEq.)		EKG Classification		Change in			
			I	F	I	F	I	F	I	F	K_{Bal} (mEq.)	K_N (mEq.)	EKG	K_S (mEq./L.)
1. G. S.	Compound F	3	4.0	3.8	0	−130	0	−75	0	0	−130	−75	⟷	.....
2. G. S.	Compound F	6	3.2	3.3	−146	−188	−80	−30	−2	−3	−42	+50	↓	.....
3. E. A.	DOCA	5	3.9	3.6	−150	−270	−130	−241	0	0	−120	−111	⟷	.....
4. A. McC.	DOCA	4	4.1	4.1	0	−61	0	−41	0	−1	−61	−41	↓	.....
5. J. McL.	Compound F	1	3.8	3.8	0	−44	0	−49	0	0	−44	−49	⟷	.....
6. H. K.†	NH_4Cl	6	3.8	4.0	0	−130	0	−130*	0	0	−130	−130	⟷	.....
7. J. M.‡	NH_4Cl	2	4.3	4.5	0	−148	0	−148	0	0	−148	−148	⟷	.....
8. R. S.	Diarrhea from laxatives	2	4.3	4.3	0	−58	0	−53	0	−2	−58	−53	↓	.....
9. F. W.§	$(NH_4)_2SO_4$	2	4.2	3.8	0	−165	0	−165	0	0	−165	−165	⟷	.....
10. W. W.	Compound F	4	4.2	4.2	0	−58	0	−22	Abn	−3**	−58	−22	↓	.....
11. W. W.	Compound F	14	4.2	4.3	−58	−233	−22	+106	−3**	−1	−175	+128	↑	.....

B. Sequences in Which Serum Potassium Decreased and Potassium Balance Was Unchanged

12. J. McL.	Compound F	3	3.8	3.2	−31	−43	−40	−32	−2	−1	.....	.....	↑	−0.6
13. G. S.	Compound F	1	3.8	3.2	−130	−146	−75	−80	0	−2	.....	.....	↓	−0.6
14. R. S.	After recovery from diarrhea	7	5.0	4.3	+32	+39	+48	+28	−2	0	.....	.....	↑	−0.7
15. W. W.	After compound F	1	4.3	3.7	−233	−199	+106	+154	−1	−2	.....	.....	↓	−0.6

C. Sequences in Which Serum Potassium Decreased and Potassium Balance Was Negative

16. H. K.	Compound F	7	4.0	3.3	0	−101	0	−48	0	0	−101	−48	⟷	−0.7

D. Sequences in Which Serum Potassium Increased and Potassium Balance Was Positive

17. R. S.	Recovering from diarrhea	3	2.7	3.3	−490	−386	−442	−332	−3	−3	+104	+110	⟷	+0.6
18. R. S.	Recovering from diarrhea	6	3.3	5.0	−318	+32	−269	+48	−2	−2	+350	+317	⟷	+1.7
19. A. B.	Recovering from diarrhea	5	2.1	2.7	−548	−408	−518	−376	−1	0	+140	+142	↑	+0.6
20. A. B.	Recovering from diarrhea	2	2.9	3.7	−297	−173	−272	−156	Abn	Abn	+124	+116	⟷	+0.8
21. A. B.	Recovering from diarrhea	5	3.7	4.8	−173	+23	−156	+29	Abn	Abn	+196	+185	⟷	+1.1
22. J. McL.	After compound F	4	3.5	4.1	−110	−7	−109	−1	−1	+1	+103	+108	↑	+0.6
23. G. S.	After compound F	3	3.2	3.8	−140	+4	+30	+210	−1	+1	+144	+180	↑	+0.6
24. A. R.††	After NH_4Cl	3	3.9	4.4	−171	−49	−171	−49	+1	+1	+122	+122	⟷	+0.5

E. Sequences in Which Serum Potassium Was Unchanged and Potassium Balance Was Positive

25. G. S.	After compound F	4	3.3	3.2	−188	−140	−30	+30	−3	−3	+48	+60	⟷	.....
26. W. W.	After compound F	6	3.7	4.0	−199	−124	+154	+233	−2	Abn	+75	+79	↑	.....
27. J. M.‡‡	After NH_4Cl	5	4.5	4.1	−148	−11	−148	−11	0	0	+137	+137	⟷	.....
28. F. W.	After $(NH_4)_2SO_4$	4	4.0	4.2	−160	+36	−160	+36	0	+1	+196	+196	↑	.....
29. F. W.	After $(NH_4)_2SO_4$	4	4.2	4.1	+36	+114	+36	+114	+1	+1	+78	+78	⟷	.....
30. R. S.	Recovering from diarrhea	2	3.3	3.3	−386	−318	−332	−269	−3	−2	+68	+63	↑	.....
31. A. B.	Recovering from diarrhea	2	2.7	2.9	−408	−297	−376	−272	0	Abn	+111	+104	⟷	.....

F. Sequences in Which Serum Potassium and Potassium Balance Showed No Change (EKG Change Only)

32. J. McL.	Compound F	2	3.8	3.8	−44	−33	−49	−41	0	−3	.....	.....	↓	.....
33. G. S.	Control	7	4.1	4.3	0	0	0	0	0	−1	.....	.....	↓	.....

Abbreviations: I = initial value
F = final value
↑ = "ascending" change (see text)
↓ = "descending" change (see text)
⟷ = no change
K_N = potassium balance, corrected for nitrogen (see text)
K_{Bal} = uncorrected potassium balance
K_S = serum potassium concentration

See text for explanation of the classification of electrocardiograms.

* K_N and K_{Bal} are identical in all the patients given acidifying salts because there was no significant change in nitrogen balance.
† Serum bicarbonate decreased from 32 mEq./L. to 17 mEq./L.
‡ Serum bicarbonate decreased from 31 mEq./L. to 15 mEq./L
§ Serum bicarbonate decreased from 30 mEq./L. to 23 mEq./L.
** Nodal rhythm; all other tracings showed normal sinus rhythm.
†† Serum bicarbonate increased from 20 mEq./L. to 27 mEq./L.
‡‡ Serum bicarbonate increased from 15 mEq./L. to 26 mEq./L.

a solid oval for concentrations between 3.0 and 3.7 mEq./L.; and an open circle for concentrations of 3.8 to 5.0 mEq./L.

It is seen in Figure 2 that there were twenty-eight instances of negative potassium balances greater than −40 mEq. Only fourteen of these showed electrocardiograms graded −1 to −3. There was no quantitative correlation between the classification of the electrocardiogram and the magnitude of the negative K_{Bal}. Of the fourteen instances with −1 to −3 tracings all but four had abnormally low serum potassium concentrations. In contrast to this in only five of the fourteen instances of potassium depletion in which the electrocardiogram showed no definite evidence of deficiency was the serum potassium concentration low. Two of these cases with hypokaliemia and normal electrocardiograms had negative potassium balances of 270 and 410 mEq., respectively. In three instances with negative potassium balances of −125 to −300 mEq. the tracings were read as nonspecifically abnormal; in two of these instances the serum potassium was low. In nine instances of potassium depletion without a deficiency tracing the serum potassium concentration was normal. Slight to moderate degrees of metabolic acidosis occurred in four of these cases as the result of treatment with ammonium chloride or ammonium sulfate. It was of interest that none of the four instances of potassium depletion produced by acidifying salts showed electrocardiographic evidences of potassium depletion and, in fact, two had tracings graded +1.

There were four instances in which the electrocardiogram was graded −1 to −3 despite the absence of significant alterations in K_{Bal} or serum potassium. In two cases there had been small acute losses of potassium due to compound F, 31 and 33 mEq., respectively. In the third instance the previously normal electrocardiogram spontaneously developed −1 characteristics during a control period of observation. The final case demonstrated the persistence of −2 characteristics after gradual correction of a severe potassium deficit had been completed. A few days after this tracing had been taken the electrocardiogram returned to normal despite the absence of further change in K_{Bal} or serum concentration.

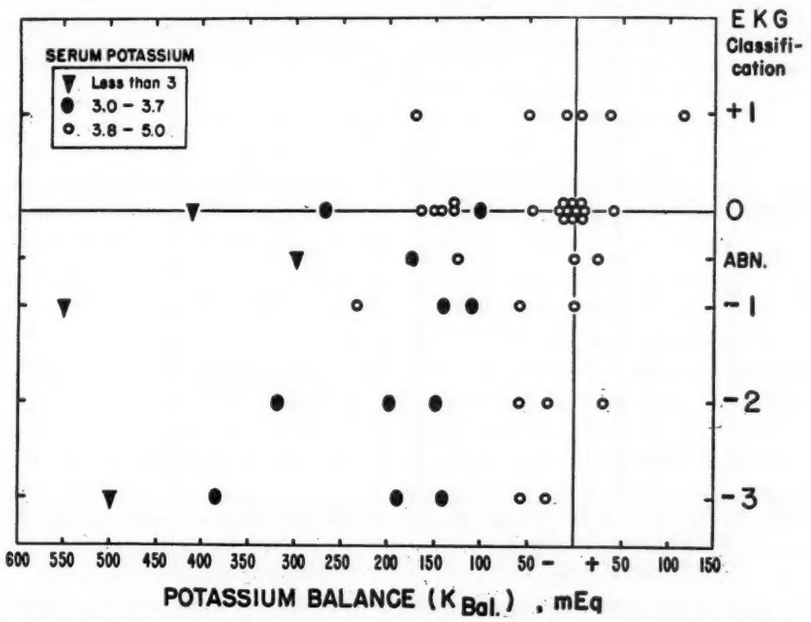

FIG. 2. The relation between uncorrected potassium balance (K_{Bal}) and the electrocardiogram.

Correction of potassium balance for changes in protoplasmic mass, using a K/N ratio of 2.7, is thought to give a more accurate indication of changes in intracellular potassium concentration.[5] In none of the present cases, except those receiving compound F, did this correction significantly alter the data. (Table I.) In Figure 3 the compound F cases are plotted for comparison both as K_{Bal} and K_N. The numbers in the circles refer to the sequences listed in Table I, and they permit visual comparison of the differences between K_N and K_{Bal}. The data plotted

in section B of the figure demonstrate a gross discrepancy between the "corrected" balance and the electrocardiogram. In seven instances in which the electrocardiogram showed definite evidences of potassium depletion, there was no significant loss of K_N and two of these cases actually had large positive balances. In section A of Figure 3 the correlation between the uncorrected balance ("K_{Bal}") and the electrocardiogram is seen to be considerably better but it is no more quantitative than that shown in Figure 2 for all the subjects as a group.

Relationship between Serum Potassium Concentration and the Electrocardiogram. In Figure 4 the points illustrated in Figure 2 are plotted with the serum potassium level as the abscissa and the electrocardiographic classification as the ordinate. The cross-hatched area between 3.8 and 5.0 mEq./L. covers the normal range for serum potassium concentration. In this figure the points are drawn in three different ways: (1) solid triangles represent instances in which the negative K_{Bal} was in excess of 200 mEq.; (2) solid ovals represent instances in which the K_{Bal} was between −40 and −200 mEq.; and (3) open circles represent instances in which the K_{Bal} was between ±40 mEq.

It is seen in Figure 4 that there is no good quantitative relationship between the degree of hypokaliemia and the electrocardiogram. This figure also emphasizes certain facts previously evident in Figure 2; (1) of fifteen instances with hypokaliemia, none of whom had acidosis, only ten had electrocardiograms graded −1 to −3; (2) of eighteen instances with −1 to −3 tracings, eight had normal serum concentrations. Four of these eight patients with normal serums and deficiency tracings had significant degrees of potassium deficiency and two others had suffered small acute losses of potassium.

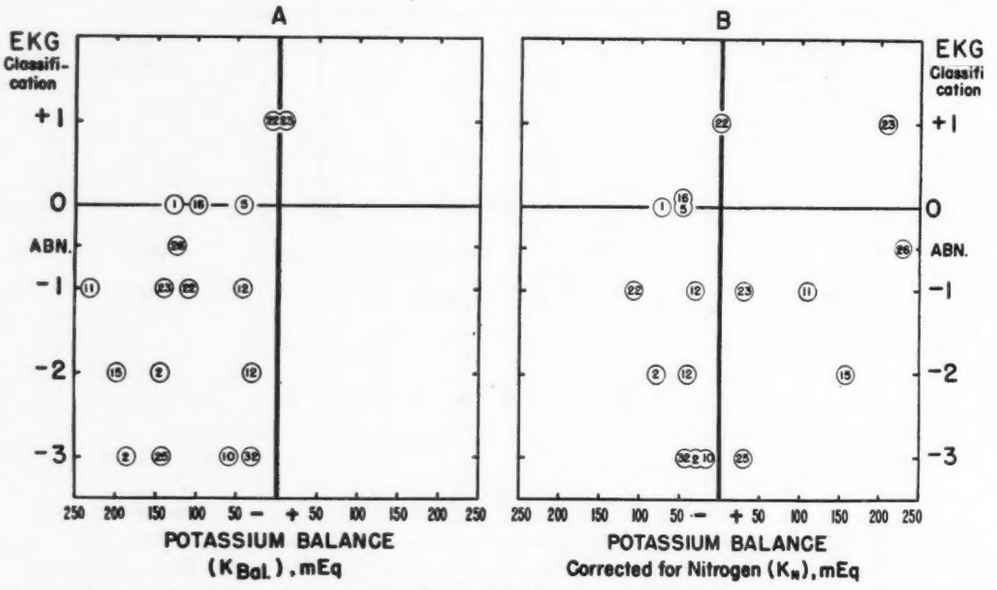

FIG. 3. The relationship of the electrocardiogram to the corrected potassium balance (K_N) and to the uncorrected potassium balance (K_{Bal}) in patients receiving compound F. The numbers in the circles refer to the sequences listed in Table 1.

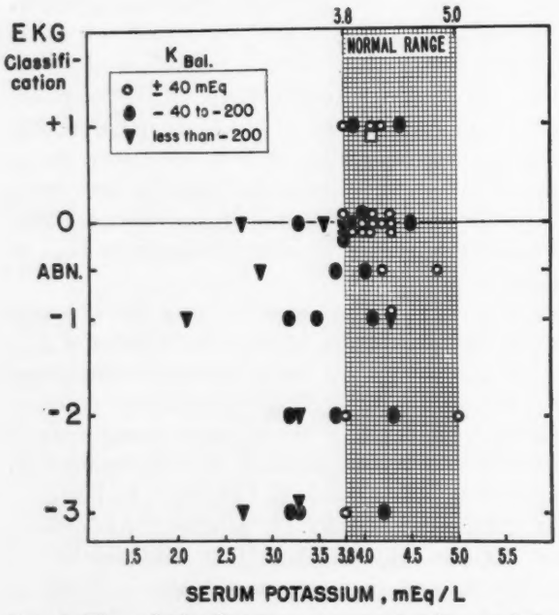

FIG. 4. The relation between serum potassium and the electrocardiogram.

COMMENTS

The data failed to show any consistent quantitative correlation between body potassium stores and the electrocardiogram. Normal electrocardiograms were found in the presence of moderately severe potassium depletion, and small degrees of depletion were sometimes associated with striking changes in the S-T-U segments.

Although Figure 2 suggests the importance of hypokaliemia in determining whether a given degree of potassium depletion will be reflected by the electrocardiogram, Figure 4 emphasizes the fact that normal electrocardiograms may occur despite low serum concentrations and, conversely, that normal serum concentrations do not always prevent the development of characteristic tracings during potassium depletion. Furthermore, it is apparent from Figure 4 that there is no quantitative correlation between the degree of hypokaliemia and the electrocardiographic classification.

The data also demonstrate that substantial retentions of potassium occurring over periods of several days during recovery from deficiency are not regularly reflected in the electrocardiogram, irrespective of whether the serum potassium concentration is concomitantly altered. Furthermore, in some instances there appeared to be a distinct lag in the electrocardiographic response to change in balance and serum concentration. For example, the electrocardiogram of patient R. S. (sequence 18 in Table 1) was unaffected by accumulation of 350 mEq. over six days which restored potassium balance and serum concentration to normal. Several days later, without further significant change in balance or serum concentration, the electrocardiogram became normal. A similar lag in electrocardiographic response was shown by patient J. McL. (sequence 5) who had lost 44 mEq. during the first day of treatment with compound F and had no electrocardiographic changes at that time. During the next two days, with no significant change in balance or serum, the electrocardiogram developed striking signs of potassium depletion (sequence 32).

It is, therefore, quite obvious that the electrocardiogram cannot be relied upon to indicate acute changes in body potassium stores even when these are large enough to be of clinical significance. Such electrocardiographic changes as do occur are not consistently related to the quantity of potassium gained or lost or to the serum potassium concentration, and the appearance of these alterations may be delayed one or more days. For these reasons it would appear hazardous to attempt to use the electrocardiogram as a daily guide to therapy of potassium depletion with potassium salts.

Failure of the electrocardiogram in potassium depletion to correlate quantitatively with the serum potassium level has been noted frequently,[8–11] and has led to the belief that the electrocardiogram may be best correlated with intracellular potassium concentration. The present observations lend no support to this hypothesis because given states of potassium balance appeared to have unpredictable effects on the electrocardiogram. Whether a negative potassium balance, although certainly an indicator of over-all cellular potassium deficiency, necessarily reflects the degree of myocardial deficiency is not yet established.[12] It is conceivable that the electrocardiogram, although not related to over-all cell composition, may be closely allied to the intramyocardial potassium concentration. The present study provides no data relevant to this question.

It is of interest to note that in most of the cases treated with compound F correction of the potassium balance figures for nitrogen balance resulted in much greater discrepancies between balance and the electrocardiogram than were observed with the uncorrected balance. (Fig. 3.) These observations suggest that the standard K/N correction is not applicable to patients treated with adrenocortical hormones.[3]

The possible significance of concomitant changes in acid-base balance and in the balance of other electrolytes deserves consideration. None of the patients made potassium-deficient by the use of acidifying salts showed deficiency patterns in their tracings and none developed hypokaliemia. Whether it was acidosis *per se*[13] or a normal serum potassium concentration[14] which prevented the development of S-T-U changes cannot be determined from the evidence at hand. Acidosis was present in only four of the instances plotted in Figure 2, and therefore can account for only a small fraction of the instances in which the electrocardiogram failed to reflect potassium deficiency. No significant changes in serum sodium, bicarbonate, chloride or calcium concentrations were noted in any of the other instances, including those receiving adrenal hormones. Fluctuations in sodium and

in section B of the figure demonstrate a gross discrepancy between the "corrected" balance and the electrocardiogram. In seven instances in which the electrocardiogram showed definite evidences of potassium depletion, there was no significant loss of K_N and two of these cases actually had large positive balances. In section A of Figure 3 the correlation between the uncorrected balance ("K_{Bal}") and the electrocardiogram is seen to be considerably better but it is no more quantitative than that shown in Figure 2 for all the subjects as a group.

Relationship between Serum Potassium Concentration and the Electrocardiogram. In Figure 4 the points illustrated in Figure 2 are plotted with the serum potassium level as the abscissa and the electrocardiographic classification as the ordinate. The cross-hatched area between 3.8 and 5.0 mEq./L. covers the normal range for serum potassium concentration. In this figure the points are drawn in three different ways: (1) solid triangles represent instances in which the negative K_{Bal} was in excess of 200 mEq.; (2) solid ovals represent instances in which the K_{Bal} was between −40 and −200 mEq.; and (3) open circles represent instances in which the K_{Bal} was between ±40 mEq.

It is seen in Figure 4 that there is no good quantitative relationship between the degree of hypokaliemia and the electrocardiogram. This figure also emphasizes certain facts previously evident in Figure 2; (1) of fifteen instances with hypokaliemia, none of whom had acidosis, only ten had electrocardiograms graded −1 to −3; (2) of eighteen instances with −1 to −3 tracings, eight had normal serum concentrations. Four of these eight patients with normal serums and deficiency tracings had significant degrees of potassium deficiency and two others had suffered small acute losses of potassium.

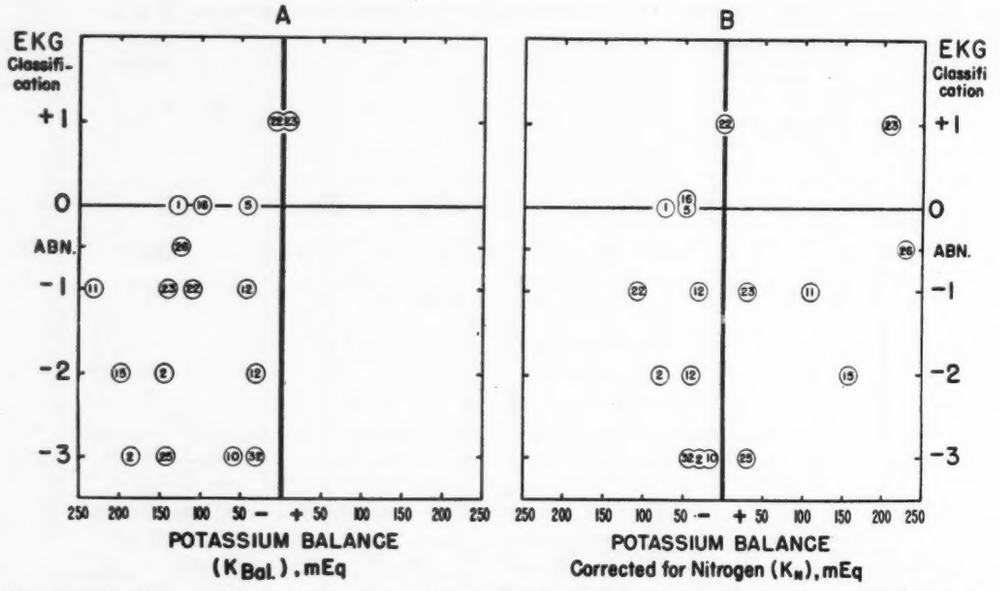

FIG. 3. The relationship of the electrocardiogram to the corrected potassium balance (K_N) and to the uncorrected potassium balance (K_{Bal}) in patients receiving compound F. The numbers in the circles refer to the sequences listed in Table I.

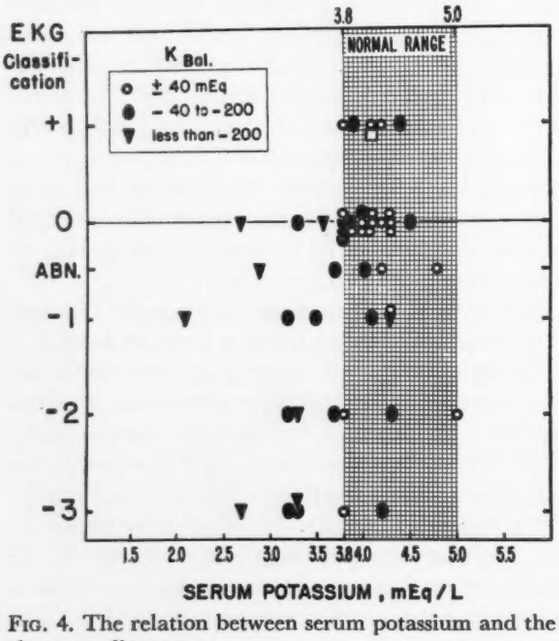

FIG. 4. The relation between serum potassium and the electrocardiogram.

COMMENTS

The data failed to show any consistent quantitative correlation between body potassium stores and the electrocardiogram. Normal electrocardiograms were found in the presence of moderately severe potassium depletion, and small degrees of depletion were sometimes associated with striking changes in the S-T-U segments.

Although Figure 2 suggests the importance of hypokaliemia in determining whether a given degree of potassium depletion will be reflected by the electrocardiogram, Figure 4 emphasizes the fact that normal electrocardiograms may occur despite low serum concentrations and, conversely, that normal serum concentrations do not always prevent the development of characteristic tracings during potassium depletion. Furthermore, it is apparent from Figure 4 that there is no quantitative correlation between the degree of hypokaliemia and the electrocardiographic classification.

The data also demonstrate that substantial retentions of potassium occurring over periods of several days during recovery from deficiency are not regularly reflected in the electrocardiogram, irrespective of whether the serum potassium concentration is concomitantly altered. Furthermore, in some instances there appeared to be a distinct lag in the electrocardiographic response to change in balance and serum concentration. For example, the electrocardiogram of patient R. S. (sequence 18 in Table 1) was unaffected by accumulation of 350 mEq. over six days which restored potassium balance and serum concentration to normal. Several days later, without further significant change in balance or serum concentration, the electrocardiogram became normal. A similar lag in electrocardiographic response was shown by patient J. McL. (sequence 5) who had lost 44 mEq. during the first day of treatment with compound F and had no electrocardiographic changes at that time. During the next two days, with no significant change in balance or serum, the electrocardiogram developed striking signs of potassium depletion (sequence 32).

It is, therefore, quite obvious that the electrocardiogram cannot be relied upon to indicate acute changes in body potassium stores even when these are large enough to be of clinical significance. Such electrocardiographic changes as do occur are not consistently related to the quantity of potassium gained or lost or to the serum potassium concentration, and the appearance of these alterations may be delayed one or more days. For these reasons it would appear hazardous to attempt to use the electrocardiogram as a daily guide to therapy of potassium depletion with potassium salts.

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chloride balance were totally unrelated to the electrocardiogram.

Certain limitations inherent in this study need comment. The data were taken from fourteen studies in eleven subjects and the number of observations chosen from each subject was arbitrary. Therefore, despite the large number of observations presented, statistical analysis of these data is clearly impossible. The data must be considered as merely illustrative of the variety of relationships which may exist between the electrocardiogram, potassium balance and the serum potassium.

Another consideration modifying interpretation of these data is that there were relatively few observations in states of severe potassium depletion and hypokaliemia. The number of instances with potassium deficits greater than 300 mEq., or serum concentrations less than 3 mEq./L. is so few that no conclusions are warranted concerning the quantitative relationship of the electrocardiogram to extreme potassium deficits. For the most part, these studies were concerned with moderate degrees of potassium depletion produced experimentally in previously normal subjects. The conclusions presented here may be applied with confidence only to those clinical situations in which comparable degrees of depletion exist.

What conclusions can be drawn from this study about the relative sensitivity of the electrocardiogram and the serum potassium in detecting potassium deficiency? The electrocardiogram was quite unreliable as an index and failed sometimes to reflect even moderately large losses of potassium. The serum potassium, while sometimes normal in the presence of slight or moderate losses of potassium, was with one exception always low in the presence of deficits of more than 200 mEq. It should be pointed out that none of these severely deficient subjects was acidotic and one cannot conclude from the present data that the serum concentration would always be reduced with severe deficits if concomitant acidosis were present.

How often will the electrocardiogram or the serum potassium concentration suggest potassium deficiency when it is not present? In these limited observations the serum potassium was never found to be less than 3.8 mEq./L. except in the presence of potassium deficiency. The electrocardiogram was misleading on only two occasions, one of which apparently represented a lag in electrocardiographic response to potassium repletion.

SUMMARY AND CONCLUSIONS

The correlation between the electrocardiogram, the potassium deficit and the serum potassium concentration was analyzed in fourteen balance studies on eleven subjects with varying degrees of potassium depletion resulting from diarrhea, or from the administration of DOCA, compound F or acidifying salts.

In only half the observations on subjects depleted of 40 to 550 mEq. of potassium did the electrocardiogram show evidence of potassium depletion. The electrocardiogram was often normal with low serum potassium concentrations and conversely was sometimes abnormal with normal serum concentrations. When changes occurred in the electrocardiogram, they were not quantitatively related to either the potassium deficit or the reduction in serum concentration. There was sometimes a lag of one or more days in the electrocardiographic response to acute loss or retention of potassium. Only in the compound F subjects were there significant losses of nitrogen, and in these cases the uncorrected potassium balance was better correlated with the electrocardiogram than was the balance corrected for nitrogen. None of the subjects with acidosis developed electrocardiographic evidences of potassium depletion.

It is concluded that in potassium depletion of moderate severity the electrocardiogram cannot be relied upon as a guide to diagnosis or therapy. The electrocardiogram is consistently related neither to the total potassium deficit nor to the serum potassium concentration. No conclusions can be drawn about the relationship of the electrocardiogram to intramyocardial potassium concentration.

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Bronchogenic Carcinoma in Young Men*

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It has been long recognized that age has a profound influence upon the incidence and behavior of neoplastic processes. For example, the greater susceptibility of various age groups to specific types of tumors and the marked increase in the total incidence of malignant diseases which occurs after the fifth decade of life are so familiar as to require no elaboration. Likewise, it is well known that those malignancies which mainly affect the young, such as neuroblastoma, Wilms' tumor of the kidney and sarcomas of various types, are characterized by a distinctive course; that is, they grow rapidly, metastasize early and cause early death. Despite a predisposition to certain types of neoplasm, youth does not render an individual immune to those new growths which occur predominantly in middle and late life. Furthermore, on the infrequent occasions when young people are affected these malignancies of old age exhibit somewhat bizarre behavior and often pursue an accelerated course. For example, carcinoma of the breast in young women is a much more menacing disease than when it occurs in later life.[1] Primary bronchogenic carcinoma in young persons behaves similarly, and it is with this specific problem that the present discussion concerns itself.

A number of impressions regarding the characteristics of primary cancer of the lung in the very young can be gleaned from the medical literature by an analysis of isolated reports of bronchogenic carcinoma occurring in children. Since the cellular makeup of the tumors was considered to be of particular interest for our purpose, only those reports were included which contained adequate histologic descriptions. Perhaps some were unjustifiably excluded, and a more diligent search of the literature might have produced a few more examples; however, it is doubtful that the observed trend would have been altered significantly. A summary of pertinent information from these cases is included in Table 1. It will be noted that, even though primary carcinoma of the lung is unusual in children, its squamous cell variant must be exceedingly rare, the collected group being composed entirely of undifferentiated tumors and adenocarcinomas. Equally impressive is the fact that bronchogenic carcinoma has been reported with the same frequency in both sexes below the age of fifteen years. Another point of interest is the average duration of life from the onset of symptoms until death. Except for two cases,[2] which had the characteristics of an adenoma, this interval was 6.1 months, in contrast to figures of 14.2[3] months, 14.5[4] months and 11.9[5] months noted in adults by various investigators.

An analysis of studies of lung cancer in younger adults shows a trend in cell type similar to that noted for children. In one large group[6] in which there were forty-six individuals less than forty years old only six had squamous cell cancers. In another study of fifty autopsied cases[7] there was but one squamous cell carcinoma in the seven patients below forty years. This incidence is considerably different from that of lung cancer as a whole, the squamous cell variety comprising approximately one-half of the cases. Thus certain trends are already known to exist but there has been little effort directed toward a more detailed analysis of the problem under consideration. In an attempt better to define these trends and to uncover other peculiarities of primary cancer of the lung in young people the following study was carried out.

MATERIAL AND METHODS

The subjects of this study were all patients below forty years of age with bronchogenic

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carcinoma admitted to the Veterans Administration Hospital, New Orleans, Louisiana, since its opening on May 3, 1946, and a similar group of males admitted to Charity Hospital of the same city since January 1, 1944. Since only men were admitted to the former institution, conclusions regarding racial incidence. Three individuals were non-smokers, five smoked less than a pack of cigarettes daily, twenty-one smoked in excess of that amount. All of the patients with squamous cell carcinomas, exclusive of one case in which no definite statement

TABLE I

REPORTED EXAMPLES OF HISTOLOGICALLY PROVEN PRIMARY PULMONARY CANCER IN CHILDREN

Reference	Sex	Age Year	Duration Life	Histologic Type
Beardsley, J. M. *Canad. M. A. J.*, 29: 257, 1933	F	10 mo.	13 mo.	Adenocarcinoma
Wasch et al.[2]	M	11	7 yr.	Adenocarcinoma (?)
Hirsch, E. F., Ryerson, E. W. *Arch. Surg.*, 16: 1, 1928	M	5	21 mo.	Adenocarcinoma
Sommer, A. W. *Minnesota Med.*, 17: 415, 1934	F	7	7 wk.	Adenocarcinoma
Thwaite, W. G. *Georgia M. A. J.*, 40: 216, 1951	M	12	9½ wk.	Adenocarcinoma
Kilduffe, R. A., Salasin, S. L. *J. M. Soc. New Jersey*, 30: 152, 1933	M	14	4 mo.	Alevolar carcinoma
Schwyter, M. *Ztschr. f. Path.*, 36: 146, 1928	F	16 mo.	2 mo.	Adenocarcinoma
Jones et al.[2]	M	13	2 yr.	Adenocarcinoma (?)
Hauser, H. *Radiology*, 39: 33, 1942	F	17 mo.	6 mo.	Undifferentiated
Cayley, C. K. et al. *Am. J. Dis. Child.*, 82: 49, 1951	F	13	5½ mo.	Undifferentiated
Dick, A., Miller, H. *Brit. M. J.*, 1: 387, 1946	F	9	9 mo.	Undifferentiated
Field, C. E., Quilliam, J. P. *Brit. M. J.*, 1: 691, 1943	F	4	2½ mo.	Undifferentiated
Lereboullet, P. et al. *Paris méd.*, 7: 145, 1936	F	5	5 mo.	Undifferentiated
LeLourd, R., Clarac, F.: *Semaine med.*, 8: 155, 1952	M	6	9 mo.	Undifferentiated
Cardelle, G. et al. *Arch. de méd. inf.*, 5: 351, 1936	M	11	3 mo.	Undifferentiated
Gaustad, A. V. *Nord. med.*, 45: 640, 1951	M	13	2 mo.	Undifferentiated

the group was made homogeneous by excluding from consideration the women with bronchogenic carcinoma from the latter hospital. A total of thirty cases, twelve and eighteen male patients, respectively, was collected from the two hospitals. In two of the Charity Hospital patients a histologic diagnosis of malignancy was never obtained and they were lost to follow-up. The clinical findings were so indicative of pulmonary cancer, however, that it is believed the data available may justifiably be included here (both had peripheral nodular lesions: in one, there was roentgenographic evidence of invasion of the adjacent ribs, and the other had a Horner's syndrome, supraclavicular and axillary lymphadenopathy and a superior mediastinal syndrome).

In evaluating the individual cases, clinical and postmortem records, roentgenograms, pathologic material and, at times, data obtained through Social Service inquiries were utilized. At this writing one individual is known to be living, twenty were followed until the time of their death, and we have lost contact with the remaining nine. Although most of the patients were white (twenty-one of thirty), it is believed that the small size of the group and the admixture from the two institutions prevent definite

was made, smoked twenty or more cigarettes daily; whereas of the three non-smokers, two had adenocarcinomas and one had an anaplastic lesion. While most of the patients were laborers, this probably represents a characteristic of the population of both institutions rather than any relationship to their cancers. An attempt was made at topographic classification of the tumors in each patient in a manner similar to that outlined by Rabin and Neuhof.[8] Since any arbitrary method of classification breaks down at times when applied to situations in which definite boundaries do not always exist and the element of human error enters, the figures to be presented cannot be accepted as definitive; however it is believed that they represent adequate approximations of the prevailing conditions. While the individual tumors could, at times, be demonstrated to contain more than one cell type, there was always a predominant cellular component. The major cell type was considered representative and was the designation used.

NATURE OF THE PRIMARY GROWTH

In sharp contrast to what is ordinarily observed, most of the primary tumors in this series were of peripheral origin. (Table II.) Of the

thirty patients studied in only six (20 per cent) did the neoplasms have their origin in the lung-root zone, i.e., from the main or lobar bronchi, in contrast to general estimates for malignant lung tumors arising in this location which range from 58 per cent[9] to 90 per cent.[10] When they did occur, these so-called root tumors were predominantly infiltrative and/or produced atelectasis, in contradistinction to the usually discrete and nodular character of the remaining growths which arose in the "parenchymal zone," that is, from segmental or smaller bronchi, or without connection with a macroscopic bronchus. Thus twenty-three (77 per cent) of the growths were situated in relation to the periphery of the lung. Twenty of these were of a circumscribed or nodular nature while the remaining three produced a segmental type of atelectasis and could not be classified as either nodular or infiltrative. Six of the nodular growths were of the Pancoast type, and three were associated with varying degrees of pleural effusion. In one case the extent of the lesion was so great as to make impossible accurate gross description of its zone of origin. It is worthy of note that in another instance a hilar lymph node focus, metastatic from a peripheral tumor, had ulcerated into a main stem bronchus and could easily be mistaken for a primary lesion upon bronchoscopic examination (Case I).

In addition to a predisposition for the peripheral portions of the lung and a circumscribed type of growth, these cancers presented interesting histologic features. (Table II.) There were only five examples (18.5 per cent) of squamous cell carcinoma in the twenty-seven cases in which the histologic features could be determined. As previously noted, this type of neoplasm is found in approximately one-half of the patients in large studies which include all ages. The remainder of our series was divided equally between the adenocarcinoma and anaplastic cell types, there being eleven of each. One case, which was diagnosed by lymph node biopsy, was listed merely as "metastatic carcinoma," and in two patients a histologic diagnosis was not available.

TABLE II

CORRELATED GROSS AND MICROSCOPIC CHARACTERISTICS OF THE PRIMARY LESION OF THIRTY YOUNG MEN WITH BRONCHOGENIC CARCINOMA

Site	Topography				Histology			
	Circumscribed	Infiltrative	Atelectatic	Unclassified	Adenocarcinoma	Anaplastic	Squamous	Unknown
Lung root	0	2	4	0	2	1	3	0
Peripheral	20	0	3	0	9	9	2	3
Unclassified	..	..	..	1	..	1	..	..
Total	20	2	7	1	11	11	5	3

In view of the atypical behavior, both grossly and microscopically, of most of the primary neoplasms in this group, one might speculate that their clinical manifestations in the affected individuals would be unusual. A detailed analysis proved such to be the case.

PRESENTING DIFFICULTIES

The complaints which are classically associated with bronchogenic carcinoma are those resulting from bronchial involvement; namely, cough, hemoptysis, wheeze and pulmonary infection. In our patients, however, these symptoms were comparatively infrequent. (Table III.) For example, a history of cough was elicited from only eighteen (60 per cent) of thirty patients in comparison to a frequency of 90 per cent cited in the recent series of Ochsner et al.[11] (Fig. 1.) Since so many otherwise normal individuals will admit to some cough, even this somewhat low incidence may represent an exaggeration and a truer impression of bronchial derangement might be obtained by an examination of other complaints. Hemopytsis was described by only four patients. Likewise, wheeze and evidences of infection, such as fever and expectoration, were initially minimal. On the other hand, evidences of involvement of the structures of the chest wall were very prominent. Pain in the chest or shoulder was the most

outstanding symptom, twenty-two patients (73 per cent) complaining of one or both. Unfortunately this figure, which is only slightly higher than that noted by Ochsner (Fig. 1), does not adequately describe the situation for it gives no indication of the intensity of the pain. by the large number of patients (seventeen of thirty) with evidences of metastatic spread to one or more structures when first examined. Hoarseness, dysphagia, venous engorgement of the upper body, and other evidences of mediastinal disease were seen in nine (30 per cent) cases.

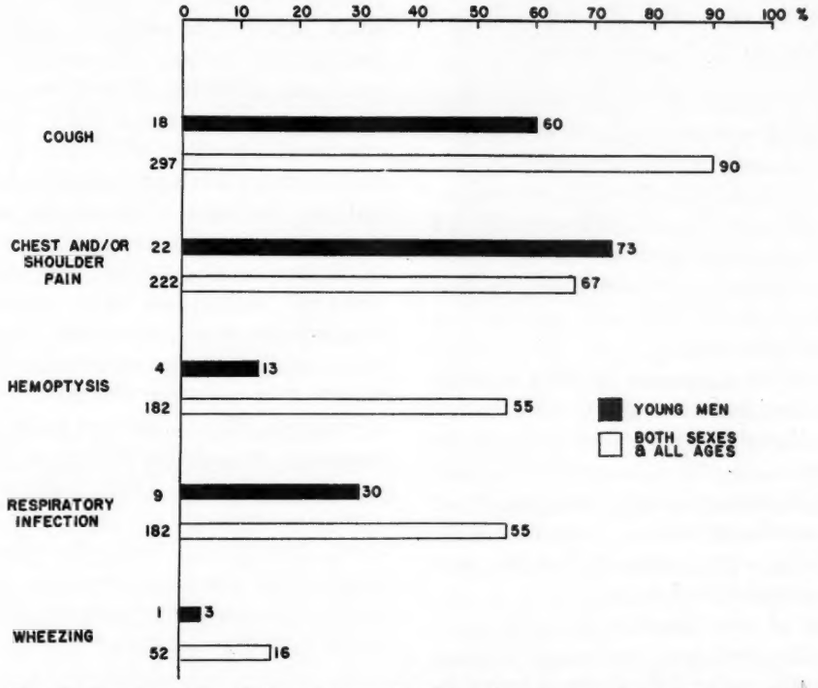

FIG. 1. A comparison of the most prominent respiratory symptoms of a group of thirty men less than forty years old and 331 patients (Ochsner et al.[10]) studied without reference to age or sex.

This was so striking in most cases that it presented the major problem of palliation. When shoulder pain was present an associated Horner's syndrome was frequently observed. Seven patients had no symptoms referable to the chest when first seen; their pulmonary disease was discovered during a routine survey or while they were being studied for some apparently unrelated disorder.

An explanation for the prominence of chest and shoulder pain, the relatively small number of patients with cough and hemoptysis, and the occurrence of many "silent" lesions, is readily derived from a consideration of the topography of the primary lesions discussed previously; namely, a preponderance of circumscribed lesions situated in the periphery of the lung away from the larger bronchi and frequently in close association to the chest wall or superior sulcus.

The threatening nature of this type of neoplasm in people below forty years is exemplified

TABLE III
PRESENTING FINDINGS IN THIRTY YOUNG MEN WITH BRONCHOGENIC CARCINOMA

	No.	Per cent
Chest and/or shoulder pain	22	73*†
Cough	18	60*
Respiratory infection	9	30
Dyspnea	9	30
Superior mediastinal syndrome	9	30
Lymph node metastases	8	27
CNS involvement	7	23
Hemoptysis	4	13
Bone pain	4	13
Pleural effusion	3	10
Horner's syndrome	3	10*
Skin metastases	2	7
Wheeze	1	3
No symptoms	7	23

* Findings of one unproven case.
† Finding of one unproven case.

Symptoms of central nervous system involvement, particularly headaches, were encountered in seven individuals (23 per cent). Eight (27 per cent) patients were found to have metastatic disease in peripheral lymph nodes shortly after admission, two had foci in the skin and four complained of bone pain.

PROBLEM OF DIAGNOSIS

At this point it should be apparent that the somewhat atypical symptom-patterns encountered, together with the youth of these patients, might present difficult problems in diagnosis. That considerable early diagnostic confusion did exist is clearly indicated by a review of initial impressions in a number of the cases in this series. Five patients were originally admitted to the tuberculosis observation unit, three were given a presumptive diagnosis of lung abscess and three were admitted for evaluation of central nervous system disturbance. Further study of the last group, however, usually disclosed respiratory findings that had been originally overlooked or minimized. A similar situation prevailed with two patients who were admitted with skin metastases of undetermined origin.

The location of the primary growths predominantly in the peripheral pulmonary zones further contributes to the difficulties in recognition by rendering diagnostic material inaccessible. Bronchoscopy, which was performed in twenty-three patients, yielded a positive biopsy on only four occasions. This examination disclosed evidences of extrinsic pressure in seven instances, tumefaction but a negative biopsy in one, mucosal injection alone in two and completely negative findings in nine. Likewise, examination of the sputum for malignant cells was of little value. Of fourteen patients studied by this method, findings were suggestive of malignancy in only two cases and completely negative in all the others. Extrathoracic lymph nodes contained metastatic lesions in eight of a total of fourteen patients subjected to lymph node biopsy. Study of a cell block of pleural fluid clarified the diagnosis in one patient. Positive evidence of malignancy was obtained from some patients by more than one of the above methods; however, in thirteen cases it was necessary to resort to surgical exploration of the thorax, and the final diagnosis was established only by postmortem examination in four instances.

TREATMENT

Fourteen patients (47 per cent) were explored but this amounted to a diagnostic procedure in many instances; and had the true nature of the process been conclusively established preoperatively, it is probable that a much smaller number would have been operated upon since many had obvious evidences of extrapulmonary disease. The tumors of six patients were resectable but in one instance it was necessary to leave neoplastic tissue behind within the chest wall. Another of the resected group underwent surgery for a presumptive lung abscess and only at the time of histologic study of the removed specimen was the true neoplastic nature of the process discovered. This individual died of cerebral metastases nine years after pneumonectomy was performed. Nineteen (63 per cent) of the group were treated with nitrogen mustard or roentgen therapy, or a combination of the two. Eleven showed little or no beneficial response, seven had a fair result, and in one there was a good effect. There were two examples of radiation fibrosis. In instances in which they were beneficial, both the chemotherapeutic agent and roentgen therapy rapidly became ineffective and there was no detectable differential response between the two.

COURSE AND PROGNOSIS

As the neoplastic process in the lungs of these patients became more widespread, with bronchial involvement and extension toward the periphery, the tendency toward unusual symptom-patterns became less apparent and the signs and complaints more closely approximated the picture of advanced pulmonary malignancy for all age groups. For example, while chest and/or shoulder pain was the outstanding admission complaint, almost all the patients eventually developed cough, and about one-half experienced some degree of hemoptysis.

This series is not large enough to warrant statistical analysis of the sites of metastatic involvement. It is worthy of note, however, that nine patients developed clinical evidence of central nervous system spread during their course, headache being the most prominent symptom. Likewise, signs and symptoms of mediastinal metastases were noted at some time in seventeen (over one-half) of the cases. Other sites of dissemination included kidneys, peripheral lymph nodes, the opposite lung, skeletal

system, adrenals, skin, liver, heart and spleen—a list not qualitatively different from figures derived from carcinoma of the lung in older age groups.

A unique aspect of the course of young persons with bronchogenic carcinoma is the rapidity with which their cancers grow, metastasize and cause death. As noted in the section on treatment, resection offered little hope of cure and there remained only a small chance for palliation. At the time of this writing all but one of the twenty-one patients who could be followed adequately have succumbed. The mean longevity of the twenty in whom the date of death is known, including the extraordinary case which survived nine years, was fourteen months. This figure does not seem remarkable when compared to the figures of 14.2,[3] 14.5,[4] and 11.9[5] months previously quoted for all ages collectively but this is somewhat misleading because of the inclusion of a few unusually long survivals. The median, which is unaffected by a few extreme values, was 7.5 months, and the most commonly occurring period of longevity, four months. The rapidity of the course becomes even more apparent when the survival periods are broken down into six-month intervals. (Table IV.) Thus it is noted that of the twenty patients who were followed until death, six (30 per cent) expired within six months of the onset of symptoms, and only five (25 per cent) survived a year or more. The one patient who is known to be alive four months after the onset of symptoms was considered inoperable and roentgen therapy has been instituted.

TABLE IV
DURATION OF LIFE OF TWENTY YOUNG MEN WITH BRONCHOGENIC CARCINOMA FROM ONSET OF SYMPTOMS UNTIL DEATH

Duration	No.	Per cent
0–5 Months	6	30
6–11 Months	9	45
12–17 Months	2	10
18–23 Months	1	5
24–30 Months	1	5
9 Years	1	5

CASE REPORTS

CASE 1. W. D., a thirty-seven year old colored male truck driver, was admitted to the Veterans Administration Hospital, New Orleans, Louisiana, on August 5, 1952, for management of a pulmonary lesion which was detected four months prior to admission on an employment survey film. At the time of the initial discovery of the abnormal shadow the patient was completely asymptomatic; however, two or three months before entry he began to have recurrent, mild, left frontal headaches and episodes of dizziness. Although further questioning elicited a history of a 15 pound weight loss, he consistently denied chest pain, cough and hemoptysis.

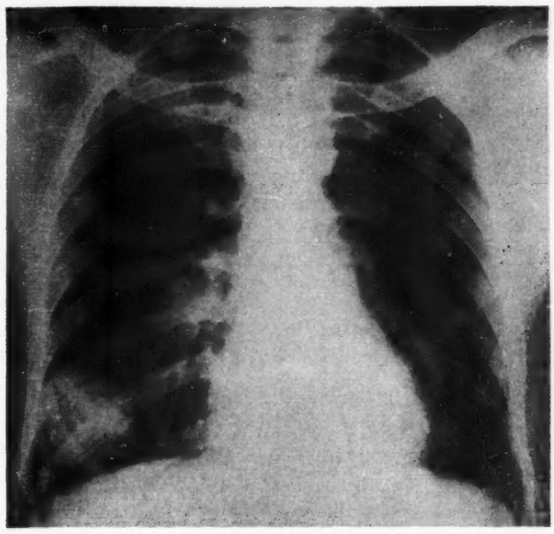

FIG. 2. Case 1. Adenocarcinoma of lung. Roentgenogram of the chest showing a peripheral circumscribed shadow in right lower lobe and hilar enlargement.

Physical examination revealed a slightly thin, colored man, who did not appear to be ill. No significant physical abnormalities could be detected. Roentgenographic studies of the chest disclosed a 6 cm. spherical, homogeneous density in the right lower lobe, and an associated nodular enlargement of the corresponding hilum. (Fig. 2.) Planigrams of the right lung confirmed these findings and, in addition, showed prominent markings adjoining the mass and hilum. There was no evidence of calcification or lucent areas within the mass. On fluoroscopy there were no detectable pulsations or change in size of the lesion with Valsalva and Mueller maneuvers. Bronchoscopy revealed only narrowing of the right main stem bronchus, apparently due to extrinsic pressure. Material obtained by bronchial lavage was negative for malignant cells. On admission, or shortly thereafter, skull films, esophagram, intravenous and retrograde pyelograms, gastrointestinal

series, electrocardiogram, hemogram, urinalysis, serologic test for syphilis, blood chemistries, liver function studies, cultures of gastric washings for acid-fast bacilli, and skin tests with tuberculin, histoplasmin and coccidioidin were normal.

After admission the patient's headaches became more severe and he developed right-sided chest pain. After nine days of hospitalization he had a transient episode of syncope. A lumbar puncture at that time revealed normal dynamics and appearance. On studies of the fluid, however, there was 92 mg. per cent of total protein, a slight increase in the globulin content and a midzone colloidal gold curve. The cell count and glucose were normal. These findings were confirmed by examination repeated four days later. At this time it seemed that the most likely diagnosis was bronchogenic carcinoma of the lung. Because of evidence of central nervous system and mediastinal metastases, it was believed that the patient was not a candidate for surgery. Consequently, a four-day course of nitrogen mustard was administered from August 22nd through August 25, 1952, following which the patient seemed to decline more rapidly. He developed exaggerated deep reflexes on the right, a right central facial weakness, funduscopic evidences of increased intracranial pressure, intermittent hiccoughs and increasing lethargy. On September 2, 1952, he became comatose and febrile, and on September 4th he died, less than four months after the onset of the initial symptoms.

Pertinent Necropsy Observations. Significant abnormalities were limited to the chest and brain. In the peripheral portion of the right lower lobe there was an 8 cm. rounded, gray-white mass, which was firm and sharply demarcated from the surrounding lung. The hilar, paratracheal and subcarinal lymph nodes on the same side were enlarged and matted together, and one of the hilar nodes had eroded into the right main stem bronchus. Microscopically, this tumor proved to be an adenocarcinoma. A metastatic focus with similar histologic findings was noted deep within the substance of the left cerebral hemisphere.

Comment. A number of pertinent points are illustrated by this case. Although the neoplasm was discovered during an asymptomatic period, the early spread beyond the confines of the lung, even prior to the appearance of respiratory symptoms, unfortunately nullified any opportunity to perform curative surgery. The peripheral situation of the lesion undoubtedly accounts for its considerable size prior to discovery and the appearance of chest pain without cough as the initial thoracic manifestations. The somewhat vague and mild nature of the first extrapulmonary symptoms demonstrates the necessity for careful inquiry and complete evaluation of all non-respiratory complaints prior to major surgery in a situation of this sort. This patient's course was one of the most fulminating we have observed.

Case II. L. V., a thirty year old white man, was admitted to the Neurological Service of the Veterans Administration Hospital, New Orleans, Louisiana, on December 26, 1950. About three months before admission he began to have intermittent frontal headaches. These became more severe and frequent, and about one and one-half months before entry there developed dysesthesia of the left arm and leg and progressive weakness of those parts. Other complaints that developed during the interim consisted of blurring of vision, loss of sense of taste and constipation. There was no history of chest pain, cough, wheeze or hemoptysis.

Physically, the patient was a hypersthenic, lethargic individual, with almost complete paralysis of the left arm and leg. Other neurologic deficits, all of which were confined almost exclusively to the left side of the body, consisted of a central type of facial weakness, exaggerated deep reflexes, diminished superficial reflexes, hemihypesthesia and a positive Babinski sign. The lungs were normal to physical examination but there was a 1½ cm. freely movable, left supraclavicular lymph node. The initial diagnostic impression was primary intracranial neoplasm in the region of the thalamus.

Views of the skull revealed pineal calcification with minimal but definite shift to the left; there was loss of definition of the right posterior clinoid. Visual field determinations showed a left homonymous hemianopsia, most marked centrally. On multiple roentgenographic views of the chest a 5 by 4 cm. lobulated density in the peripheral portion of the left upper lobe was noted, with associated nodular hilar enlargement. (Fig. 3.) Planigrams failed to disclose evidence of bronchial derangement or areas of breakdown. Fluoroscopy with barium swallow was non-contributory. Electroencephalogram, intravenous pyelogram, blood chemistries, hemogram, urinalysis, liver function studies, serologic

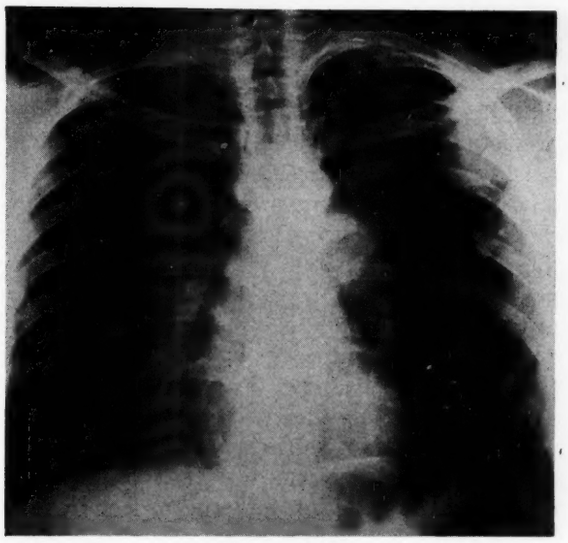

FIG. 3. Case II. Undifferentiated carcinoma of lung. Roentgenogram of the chest showing mass in peripheral portion of left second and third anterior interspaces and enlargement of corresponding hilar structures.

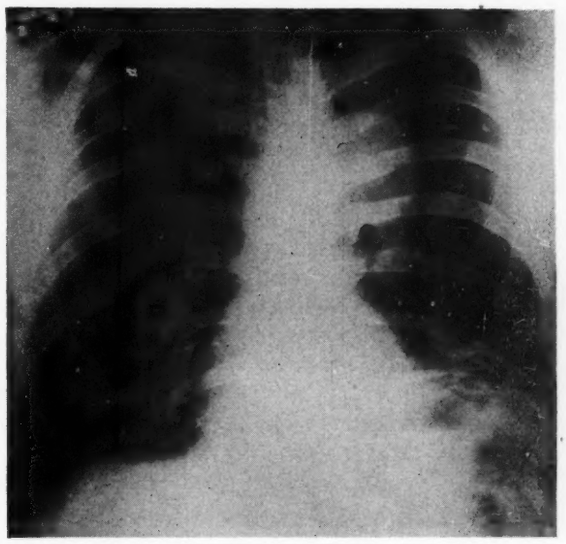

FIG. 4. Case III. Undifferentiated carcinoma of lung. Roentgenogram of the chest showing a circumscribed left paramediastinal mass.

test for syphilis and study of three sputum samples for malignant cells were normal.

When it was learned that the patient had a pulmonary lesion, it seemed likely that the intracranial condition represented metastatic disease secondary to a primary lung tumor, and a tentative plan to perform a craniotomy was abandoned. The enlarged left supraclavicular node was biopsied on January 4, 1951, and found to contain metastatic undifferentiated carcinoma.

The patient's course was one of rapid deterioration. He required frequent analgesics for headaches, became progressively more lethargic, and developed recurrent projectile vomiting, urinary incontinence and cough. On January 16th he had a generalized convulsive seizure. Nitrogen mustard was administered on the following two days but was discontinued after that because of the hopelessness of the situation. Papilledema and Cheyne-Stokes respiration were observed. Finally, the patient became stuporous, developed a rectal temperature of 107°F. and died on January 20th, less than four months after the onset of symptoms.

Pertinent Necropsy Observations. In the periphery of the upper lobe of the left lung there was a firm, gray-white tumor mass, measuring 10 cm. in diameter; the center was slightly necrotic. The hilar lymph nodes of the corresponding side were enlarged, firm and matted together. Likewise, the nodes in the left axilla, left supraclavicular and infraclavicular areas, and the left side of the neck appeared to be involved with neoplastic tissue. The microscopic diagnosis was undifferentiated carcinoma of the left lung with metastases to the sites mentioned above. Additional pathologic diagnoses were pulmonary edema and congestion, cardiac hypertrophy, congestion of the spleen and fatty metamorphosis of the liver. Unfortunately, the central nervous system could not be examined but the character of the clinical course leaves no doubt regarding the presence of metastases to the brain.

Comment. This case re-emphasizes many things previously noted in the first example presented. The dominance of the central nervous system symptoms, the peripheral type of growth and the fulminating course, were common to both. In this latter instance neurologic symptoms prompted admission to the hospital; and because of the age of the patient and the absence of pulmonary complaints, the discovery of a primary carcinoma of the lung was a surprise. The presence of metastatic disease in an accessible lymph node further illustrates the menacing nature of this disease in the young and the need for a meticulous search for extrapulmonary foci.

CASE III. H. Y., a twenty-seven year old white man, was admitted to the Orthopedic Service of the Veterans Administration Hospital, New Orleans, Louisiana, on November 24, 1951, for treatment of recurrent dislocation of both shoulders. He described mild pain in the

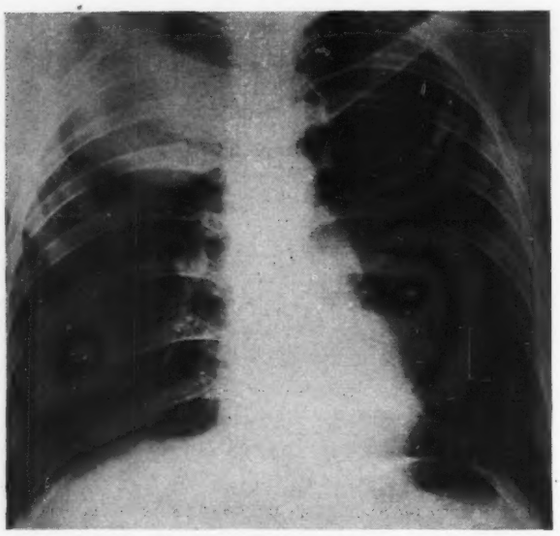

FIG. 5. Case IV. Adenocarcinoma of lung. Roentgenogram of the chest showing a large mass in right upper lobe.

left shoulder which he attributed to shoulder dislocation incurred several weeks before. There was no history of cough, hemoptysis or wheeze.

Physical examination revealed slight muscular atrophy of both deltoid regions, guarded movements of both shoulders and bilateral pterygia but no other abnormalities. Hemogram, serologic test for syphilis and urinalysis were essentially normal.

Shortly after admission the patient was subjected to a capsulorrhaphy of the right shoulder before the report of the initial chest roentgenogram had been received. That examination was later found to show a well defined, 7 cm. mass in the paramediastinal region of the left upper lobe. (Fig. 4.) On planigraphs this lesion was noted to be separate from the mediastinum and there was no definite distortion of the bronchial architecture. Fluoroscopically, it was determined that pulsations were present, apparently transmitted from the adjacent pulmonary artery. The left hemidiaphragm was elevated. Bronchoscopy showed encroachment on the trachea along its left lower lateral aspect but the mucosa of the bronchial tree appeared normal. Study of bronchial washings and a single sputum specimen for malignant cells was negative. Because of the uncertain nature of the lesion, thoracotomy was recommended; however, the patient deserted the hospital on December 20, 1951, before the procedure could be carried out.

On December 27, 1951, the patient sought readmission to the hospital. At that time his left shoulder pain was described as being more severe. Furthermore, in retrospect it was learned that he had minimized the pain during the previous admission since he feared he might have the same disease as his father, who died of lung cancer. On January 2, 1952, a left thoracotomy was performed and a large mass about the size of a tennis ball was found in the midportion of the left upper lobe, with extension to the mediastinum above the arch of the aorta. Several mediastinal nodes were noted to be enlarged, firm and fixed. Frozen sections were consistent with undifferentiated carcinoma and permanent sections subsequently confirmed this impression. The patient was not considered a candidate for resection and the procedure was terminated. Following surgery he frequently required analgesics for sharp pains in the shoulder and chest. Serial roentgenograms revealed gradual enlargement of the cancer. The pains responded moderately to a course of therapy with nitrogen mustard but recurred a short time later. Roentgen therapy was contemplated but again the patient deserted the hospital on March 6, 1952.

It was learned from the patient's wife, through the efforts of Social Service, that he died on October 21, 1952. Terminal symptoms consisted of severe cough with expectoration of purulent sputum, severe chest pain, dyspnea, hoarseness, weight loss, fever, weakness, bone pain, upset stomach and general swelling. At no time did he complain of headache or hemoptysis.

Comment. The topographic characteristics of the growth in this patient who, incidentally, was one of the youngest in our study, is consistent with the early appearance of shoulder pain in the absence of bronchial symptoms and our inability to establish a diagnosis by bronchoscopy. A thoracotomy was necessary in view of the obscure nature of the lesion; and in the presence of mediastinal metastases, that procedure accomplished nothing more than resolution of the problem of etiology. That extension of a peripheral neoplastic focus is associated with a loss of the initial atypical symptom-complex is shown by the eventual appearance of findings referable to both chest wall and bronchial involvement.

CASE IV. H. M., a thirty-three year old white man, was admitted to the Veterans Administration Hospital, New Orleans, Louisiana, on April 25, 1951. In December, 1950, he developed a

non-productive cough. In February of the following year roentgenogram of the chest disclosed an abnormal shadow in the right lung. Two months before entry the patient developed constant pain over both suprascapular areas, more marked on the right, and with radiation down the right arm. Other symptoms consisted of a steady, gradual weight loss, transient anterior chest pain, progressive dyspnea and recurrent throbbing pain in the hips and lower extremities.

The patient appeared pale and chronically ill. There was decreased resonance over the right upper anterior chest region and diminution of breath sounds and fremitus in the same area. A poorly defined tenderness was elicited over the suprascapular and upper chest regions. Roentgenograms of the chest revealed a 9 cm. rounded, discretely outlined, homogeneous density in the right upper lobe, separate from the mediastinum. (Fig. 5.) Superolaterally, this mass blended with a localized area of pleural thickening. A fibrocalcific density of questionable significance was noted in the left subapical region. On planigraphic study of the right lung the upper lobe bronchus appeared normal but all of the segmental bronchi were obstructed; no mediastinal or hilar enlargement was detected. Skin tests with histoplasmin and first-strength tuberculin were positive. Skull films, five sputum studies for malignant cells, cerebrospinal fluid examination, hemogram, urinalysis, blood chemistries, and sputum studies for acid-fast bacilli and fungi were normal. On bronchoscopy, thick secretions were noted in the right upper lobe bronchus and there was edema and injection of the mucosa around the orifice of that bronchus.

On May 16, 1951, a right thoracotomy was performed. A large tumor was found to involve the greater portion of the right upper lobe and it was densely adherent to the chest wall. Although the mediastinal nodes were grossly involved, the mass was dissected away from the chest wall and a "palliative" pneumonectomy performed. Histologically, the lesion proved to be an adenocarcinoma.

The patient was discharged from the hospital on June 4, 1951. On September 4, 1951, he was admitted to another institution complaining of pain in the left side, lumbar area and left leg. Following a generalized convulsion on September 9, 1951, he had loss of speech and a right hemiplegia. He died three days later, nine months after the onset of symptoms.

Comment. This type of lesion associated with symptoms of brachial plexus involvement is typical of a situation encountered in a number of our cases. Despite its large size, the growth was not amenable to bronchoscopic biopsy and the need for a major surgical procedure to clarify the etiology is again seen. The involvement of the mediastinal nodes with neoplastic tissue and the terminal neurologic symptoms demonstrate a pattern of extrapulmonary spread which was exceedingly common in our cases.

OBSERVATIONS

This study discloses, in brief, that the main items of difference between these young men with cancer of the lung and other series which are composed predominantly of people in middle and late life, are (1) a preponderance of peripheral and circumscribed growths, with coincidental symptomatic and diagnostic peculiarities; (2) a relatively small number of the squamous cell variant and (3) a course characterized by rapid growth of the primary process with early metastases and death. In our efforts to comprehend the underlying factors which might be responsible for these differences, we have utilized both factual and speculative data.

The rapidity of the course may be accounted for, in part, by the predominance of undifferentiated and glandular malignancies, inasmuch as these tumors are characteristically associated with a more rapid type of growth, even in old people. Another factor which is also worthy of consideration is the youth of the involved tissues. Since propensities for regeneration and reparation are inherent traits of normal tissues in the young, it may be that an exuberance of growth in their malignant counterparts represents a reflection of these normal intrinsic qualities.

An attempt to understand the topographic and histologic differences of lung cancers occurring in young and old people might profit from a consideration of some of the proposed etiologies of pulmonary malignancies. Most studies have centered around various aspects of chronic irritation; and in view of recent reports, it seems that there can be little doubt about the importance of such irritants as tobacco-smoke in the general population,[10] and asbestos[12] and the chromates[13] in select groups. This approach, however, fails to explain adequately the occurrence of primary lung cancers in the extremely young, since the interval of exposure to possible noxious agents seems short. It has been estimated, for example, that the carcinogenic effects of

smoking appear predominantly after the age of forty-five,[10] while the average duration of exposure in lung cancer occurring in asbestos and chromate workers was fifteen[12] and 10.6[13a] years, respectively. The equal involvement of the two sexes in childhood, in contradistinction to the marked predilection for men of advancing years, likewise bespeaks different causal mechanisms. Graham,[14] as well as Ochsner and coworkers,[11] who are among the leading advocates of an etiologic relationship between tobacco and carcinoma of the lung, have attempted to obviate some of the deficiencies of that theory by proposing that smoking predisposes to the epidermoid variety (and also some tumors classified as undifferentiated), while adenocarcinoma has a distinctly different etiology, i.e., it arises from congenitally maldeveloped bronchial buds and is not particularly influenced by chronic irritation. Because of the embryonic origin of the latter, they further believe that such tumors are likely to become evident earlier in life and occur with about equal frequency in both sexes. Whether their concept of the origin of the glandular growths is correct or not, our study confirms the belief that they are more frequently encountered in young people. Moreover, since the relative and actual incidence of the squamous variety increases in old people and, as noted by two careful English workers,[10] the risk of developing bronchogenic carcinoma increases in simple proportion with the amount smoked after the age of forty-five, it is tempting to accept the contention that smoking is most intimately associated with epidermoid cancer of the bronchi.

The tendency of the bronchial epithelium to respond by squamous metaplasia to irritative processes, such as chronic infectious states and the pneumoconioses, is well known; and many workers, both in clinical[15] and experimental[16] circumstances, have believed that they could follow a transition from this type of proliferation to malignant degeneration within the confines of the basement membrane and, finally, to frankly invasive carcinoma. It is worthy of note that such an association of changes occurs most commonly in the larger bronchi, and that the type of cancer observed in these situations has been, with few exceptions, the squamous variant, a type which is uncommon in young adults and not encountered in our review of cases in children. A greater effect of external irritants on the major bronchi would account for the preponderance of lung-root tumors, chiefly of the squamous-cell type, seen in old individuals, as compared with the young.

It will be noted that we have neglected to discuss the position of the undifferentiated cell type of neoplasm in these postulates of cause and effect. Since many authorities include some undifferentiated tumors in the adenocarcinoma class, while others believe that many are immature variants of squamous cell carcinoma, it seems likely that this group represents, for the most part, a wastebasket for those growths which cannot be designated as one of the other types because of inadequate differentiation on the part of the body and/or defects in our diagnostic methods. Their uncertain position, therefore, does not render them amenable to this type of study.

SUMMARY

1. In a study of bronchogenic carcinoma occurring in a group of thirty men less than forty years of age, it has been found that twenty-three, or 77 per cent, of the primary lesions were peripheral in location whereas only six, or 20 per cent, were situated in the lung-root zone. (One lesion was too extensive to warrant classification.) Twenty of the peripheral lesions were nodular in nature and only three produced atelectasis.

2. In twenty-seven of the patients in whom the histology of the growths was established, there were eleven adenocarcinomas, eleven undifferentiated tumors and only five squamous cell cancers.

3. The presenting symptom-patterns, bronchoscopic results and cytologic studies of the sputum were consistent with the high incidence of peripherally situated neoplasms. Twenty-two patients (73 per cent) described chest and/or shoulder pain, usually of a severe degree, eighteen (60 per cent) had cough, and only four had experienced hemoptysis. Seven patients had no pulmonary symptoms on admission. Bronchoscopy was performed in twenty-three individuals but yielded a positive diagnosis on only four occasions. Of fourteen patients whose sputum was studied cytologically, the findings were negative in twelve instances and suggestive of malignancy in two.

4. Because of the inaccessibility of biopsy material, the diagnosis was established by exploratory thoracotomy in thirteen cases and by necropsy in four.

5. The rapidity of growth and spread of pulmonary cancer in young people is reflected in a number of facts: seven patients had evidences of central nervous system metastases when first seen, eight had foci of tumor in peripheral lymph nodes and, although fourteen cases were subjected to exploration, the tumors in only six were resectable. This procedure was curative in no known case. The average duration of life from the onset of symptoms until death was fourteen months, but of greater significance in this comparatively small series was the median value of 7.5 months and the fact that only 25 per cent of the patients lived longer than a year.

6. These observations, as a whole, represent a marked contrast to the manifestations of bronchogenic carcinoma in old people, and it is suggested that these variations represent the effect of different causal mechanisms.

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Review

Studies of Ulcerative Colitis*

II. The Nature of the Somatic Processes and the Adequacy of Psychosomatic Hypotheses

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SINCE the first report by Murray in 1930 an impressive amount of literature has accumulated which supports the concept that psychologic influences play a significant role in the course of ulcerative colitis. Contributions to our knowledge of these relationships have come from internists, gastroenterologists, psychiatrists and psychoanalysts. Notable among these have been Alexander, Daniels, Grace, Lindemann, Murray, Paully, Portis, Prugh, Sperling, Sullivan, Szasz, Wittkower, Wolf and Wolff. Each of these workers has contributed new knowledge based on the particular frame of reference within which they have worked. But, as is especially true in psychosomatic research, involving as it does more than one conceptual scheme, no investigator has found it possible to apply the same precision of observation and conceptualization to all aspects of the disorder. This weakness has been most evident in the various hypotheses that have been offered to explain the relation between the psychologic and somatic processes. It is obvious that no hypothesis can be considered valid which does not correctly describe and identify both the basic pathologic processes in the bowel and the basic psychologic phenomena. It is our purpose in this review to examine the available data on the nature of the somatic process and to consider how adequately the various hypotheses so far advanced account for the observed facts. In a second review we will analyze the psychologic data and at that time attempt to construct an hypothesis to fit the facts as currently known.[1a]

I. CLINICAL OBSERVATIONS

In achieving understanding of any disease process the first requirement is careful and accurate clinical observation. Ulcerative colitis is a disease which occurs with about equal frequency among men and women,[2,5,7] although our own material collected since 1945 shows a preponderance of women.[1b] The disease may begin at any age and cases have been reported among newborn and infants[3,7] as well as among the aged.[2] In our series of thirty-two patients the age of onset ranged from two to fifty-three years.[1b] The majority of cases begin before the fourth decade.

A. Mode of Onset. The most promising method of inquiry to elucidate pathogenesis is a painstaking examination of the sequence of events leading up to the full-blown disorder. Once the pathologic processes in the bowel have become well established the situation becomes too complicated to permit identification of the primary psychophysiologic mechanisms. Such factors as secondary infection or fibrosis not only are unrelated to the psychologic processes but may also result in altered behavior of the bowel in response to psychologic factors.

Eliciting this kind of information from patients is more difficult than might be anticipated. Since most patients, as well as physicians, equate colitis with diarrhea, what is usually obtained is the time of onset of diarrhea. When the patient is carefully queried as to the first deviations from their usual bowel activity a somewhat different picture emerges. The results of such an inquiry among thirty-two patients is published elsewhere.[1b] In twenty-two cases (68 per cent) the earliest abnormality was the passage of blood; in ten it accompanied constipated stools, in six normally formed stools and in six diarrheal stools. Diarrhea was the presenting symptom in only twelve patients (37 per cent), twenty pa-

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tients having constipated or normally formed stools at the onset.

Of the fourteen patients who were constipated at the onset five developed diarrhea in a matter of days, seven did not develop diarrhea for months and two remained constipated even after a year. Ten of the constipated patients passed blood from the start while four had sudden development of such severe constipation, usually with cramps, that they took cathartics or enemas, following which diarrhea and bleeding quickly developed.

The order of development of symptoms during a relapse could be established with certainty in seventeen patients. Thirteen reported as the first abnormality the passage of fresh blood with formed stools, two reported bloody diarrhea and two reported diarrhea without gross blood.

The prominence of bleeding rather than diarrhea as the presenting symptom is not generally recognized. While most writers on the subject are quite aware that the disorder may begin with bleeding without diarrhea, the general conception in the literature stresses diarrhea as the initial symptom and this has been carried over into many psychosomatic formulations (see references[1a]). The one exception is Bargen[2] who states that of 871 patients with "thrombo-ulcerative colitis, type I," 50 per cent said their illness began with the passage of one or more bloody rectal discharges without other apparent symptoms.

B. Symptoms and Degree of Bowel Involvement. In a recent study of twenty-eight patients an attempt was made to correlate the degree of involvement of the bowel as revealed by radiographic and sigmoidoscopic examination with the character of the symptoms at the time of examination.[1b] The results showed that diarrhea is associated with more widespread involvement of the bowel. When the disease is confined to rectum or rectosigmoid there may be frequent passage of blood, pus and mucus but stools may remain normal in consistency or even continue to be constipated. When the disease involves segments proximal to the sigmoid or when more than the distal half of the descending colon is affected, diarrhea is a much more consistent symptom.

It would appear that when enough of the colon is involved diarrhea results from hastened peristalsis, diminished absorption and exudation through the bowel wall. On the other hand, when the process is confined to the rectum or rectosigmoid there may be a protective spasm so that feces do not enter the involved area and constipated stools result, with tenesmus, rectal spasm and the passage of blood, mucus or pus. Bargen[2] and Palmer[4] have reached similar conclusions.

C. Pre-morbid Bowel History. There is a general impression that most patients with ulcerative colitis have had bowel disturbances prior to the onset of the colitis. Of twenty patients twelve reported chronic constipation, either severe or moderate. In all of these frequent use of cathartics and/or enemas had been considered necessary. In most of them this had been initiated in childhood by a parent and continued by the patient in adult life. Six patients described normal bowel activity in the past although three of these revealed excessive parental attention to their bowel behavior, including training before one year, and one man mentioned several episodes of severe constipation associated with painful rectal spasm. Two patients described brief episodes of diarrhea when "tense."[1b]

D. Influence of Operations on the Bowel. It is general clinical experience that following short-circuiting operations active disease, including hemorrhagic phenomena, may persist or recur in the by-passed colon. Further, we have recently observed three patients who, following ileostomy and partial or total colectomy, developed ulcerative ileitis. In all three cases a small segment of terminal ileum was removed at operation and demonstrated to be normal.[1b]

Comment. The clinical data are important in calling attention to some process, which is characterized by bleeding, as the basic phenomenon in ulcerative colitis. They also indicate that the character of the fecal evacuations is dependent on the severity and location of this process and that in some instances neither diarrhea nor constipation can be interpreted in psychologic terms. Both of these facts indicate that the process primarily involves the mucosa and/or submucosa and that the bowel is responding to this rather than functioning as an integrated whole. The surgical observations also reveal that this process is not dependent on continuity with the small bowel or on small intestinal content and that identical changes may occur in the ileum after it has been separated from colon.

II. PATHOLOGY

In 1949 Warren and Sommers reported their findings in a series of 120 surgically treated and

sixty autopsied cases of ulcerative colitis.[5] As is generally accepted, they found the sigmoid most often involved, the descending colon next, then the transverse colon and rectum, and the cecum least often. The ileum was involved in one-third of the cases. Changes in the mucosa and submucosa were the most important. Grossly, there were marked hyperemia and congestion of the mucosal surface, petechial hemorrhages and the characteristic ulcers, which at times were so broad and coalescent that mucosa was practically absent from all or part of the colon. Nineteen cases had extensive involvement of blood vessels, such as necrosis of the vascular walls, thromboses, arteritis, phlebitis or periarteritis. Medium-sized submucosal arteries were affected uniformly and in the early stages the overlying mucosa appeared unchanged. Seventy-one cases showed crypt abscesses. In these the earliest lesion was the appearance of polymorphonuclear leukocytes within the lumen of individual mucosal crypts just above their bases, with no abnormality of the epithelium. With further accumulation in the affected crypt the abscess ruptured, spilling into the submucosa and dissecting beneath the epithelium, which finally ulcerated. These lesions were superficial and appeared to irritate the deeper layers of the colon without damaging them. In ninety cases the pathologic changes were so extensive or of such long duration that it was impossible to decide how they developed.

In the discussion of their findings Warren and Sommers point out that the chief manifestations of ulcerative colitis are seen in and just beneath the mucosa. They express doubt that spasm of the colon could produce ulceration. They were unable to find support for the observation of Lium and Porter[6] that the ulcers lie in relation to taenial muscle bands. They noted the hypertrophic changes to occur in the circular muscles and the muscularis mucosa, and suggest that they result from spastic contraction secondary to mucosal ulceration. The occurrence of crypt abscesses suggested to them the possibility that some substance is being secreted or excreted into the affected crypts. While they believe that chronic bacillary dysentery and ulcerative colitis are pathologically distinct, they mention that other authorities consider the pathologic changes in these two disorders to be indistinguishable. But they emphasize that the early lesions they describe in ulcerative colitis are not specific. Vasculitis may accompany bacillary dysentery, mesenteric thrombosis or volvulus, while crypt abscesses have been found in amebic dysentery, lymphopathia venereum and vitamin deficiency. Because crypt abscesses and local vasculitis were the most common precursors of ulceration they suggest the possibility of a circulating chemical agent affecting the colon and point out the release of such substances during emotional stress is not inconceivable.

Lium and Porter[6] found in six cases that the most severe lesions occurred in the rectum and that above the rectum the ulcers were linear in distribution and were located over the taenial bands. They suggest that the distribution of lesions over the most powerful muscles of the colon may be related to hypermotility and spasm of the colonic muscles. Monaghan[7] also found some tendency for the ulcers to follow the distribution of both longitudinal and circular muscles and Reiner[8] finds a longitudinal arrangement along taeniae coli in about 10 to 20 per cent, usually in extending active regions. All agree that in the rectum the ulcers tend to be totally irregular in location. Monaghan believes the thickening of the muscular layer is due to edema rather than to hypertrophy and emphasizes that in advanced stages muscle may be replaced by fibrous tissue. Levine et al.[9] have recently reported changes in the ground substance of the basement membrane of the epithelial cells. Such changes are thought to be associated with the hypersensitive state.

Comment. Morphologic data are important but still of limited value because specimens usually are available only after the disease has become well established. Therefore the description by Warren and Sommers of the steps in the development of the lesions does not necessarily correspond with the sequence of events initiating the pathologic changes. Nonetheless, the pathologic findings are important in locating the major changes in mucosa and submucosa. While most authorities agree that there are differences in the pathologic characteristics of bacillary dysentery (i.e., the lymphoid hyperplasia), nevertheless in certain basic ways the pattern is very similar, suggesting that in all these conditions the category of responses observed is that characteristic of noxious agents of molecular size.

III. SIGMOIDOSCOPY

Sigmoidoscopic observations at various stages present a more dynamic picture of the development of the disorder. Monaghan's description of

the early changes is as follows:[7] The earliest change is a mottled discoloration due to a shower of petechiae under the mucosal surface. Evidences of edema are minimal. The mucous membrane may show a few fine bleeding points. This appearance may be replaced in a matter of twenty-four to forty-eight hours or may persist for weeks or longer. The next changes consist of marked general hyperemia and the mucosa may appear somewhat swollen. It has a moist, glistening character and there is excess of mucoid secretion. The surface, after swabbing, looks granular and shows frank bleeding points. Ulcerations, if present, are usually small and shallow. This picture may persist for months but usually progresses within a week or more to a stage where the mucosa is red and edematous, showing the characteristic pitted, granular appearance. Usually there is a constant oozing of blood from the surface. Ulceration is extensive and variable as to size and depth, with little or no normal-appearing mucous membrane. There may be thick, mucopurulent exudate. At this stage there may be excessive spasm.

Comment. Of note in this description is that submucosal and mucosal vascular changes precede the development of ulceration and severe spasm.

IV. DIRECT OBSERVATIONS OF COLON ACTIVITY IN PATIENTS WITH ULCERATIVE COLITIS

Grace, Wolf and Wolff[10] report observations on two patients with ulcerative colitis in whom direct observations could be made of cecum and ascending colon which had prolapsed through a cecostomy. These investigators reported that life situations provocative of "abject fear and dejection" were associated with hypofunction of most of the large intestine with pallor, relaxation, lack of contractile activity and relatively low concentrations of lysozyme in the colonic secretions. On the other hand, life situations provocative of conflict with feelings of "anger, resentment, and hostility," or of "anxiety and apprehension" were found to be associated with hyperfunction of the colon, manifested by increased rhythmic contractile activity and ultimately by intense and frequent waves in the cecum and ascending colon and replacement of rhythmic activity on the left by sustained contraction of longitudinal muscles with shortening and narrowing of the colonic lumen. Thick, tenacious mucus secretion was observed during phases of anger and resentment while a thinner, more watery secretion was associated with periods of relative tranquility. Sustained hyperfunction was associated with the appearance of petechial lesions and an increase in the fragility of the mucous membrane. In one subject on two occasions a period of protracted resentment resulted in intense hyperfunction, leading to mucosal erosions and ulcerations. Of great importance is the fact that this man had an ileostomy and hence the lesions developed in bowel not bathed by intestinal content.

Two other fistulous patients, without ulcerative colitis, showed changes of the same order but less intense and less prolonged. The mucosa was much less often red and the colon less often hypercontractile than in the two subjects with ulcerative colitis.

Winkelstein reports additional observations on the second of the two patients studied by Grace.[11] He stressed the extreme and rapid fluctuations in the color of the mucous membrane, ranging from marked pallor to severe hyperemia, even to petechial hemorrhages, in association with certain emotional changes, while secretion, mucosal fragility and lysozyme varied in narrow limits and motor activity was markedly diminished.

Wener and Polonsky report observations on a twenty-one-year old woman with ulcerative colitis for seven years.[12] The patient had had a left hemicolectomy and a transverse colostomy, leaving the distal portion of diseased sigmoid and rectum intact. The proximal colon was healthy. Observations were made of the exposed collar of colonic mucosa. On experiencing "resentment" when the doctor performed or threatened a painful injection, the mucosa became engorged but motility ceased. When the patient was "resigned" to the experiment there was no engorgement. When pain was produced by the application of a clamp to the head and sustained for longer than anticipated, the inactive, engorged bowel now became hypermotile, with increased frequency and amplitude of contractions. The mucosa became hyperemic, engorged, edematous, friable, bled easily and scattered petechiae appeared. When resentment persisted after the clamps were removed the engorgement also persisted, although with transient blanching of the mucosa and inhibition of motility. During a five-month period the investigators made frequent observations of the colon and attempted to correlate this with the patient's emotional state. When the authors considered the patient

to be "depressed, angry, or resentful," engorgement and hyperemia were more pronounced.

Since there were also changes in skin color, sweating, pulse rate and blood pressure accompanying the reactions in the colon, it was believed that the colonic changes were part of a generalized bodily reaction to emotional stress. When the subject complained only of an intense pain in an unemotional manner or when fear was artificially produced, the reaction was "a generalized sympathetic response with blanching of the mucosa, inhibition of colonic contractions, pallor, sweating and tachycardia." In other circumstances mixed reactions were observed which the authors believed could not be readily correlated with the psychic state.

Wener and Polonsky observed that when there was prolonged hyperemia and engorgement of mucosa it became edematous and friable, and scattered petechiae occurred. Then vigorous contractions led to minute superficial hemorrhages. They believed that both sympathetic and parasympathetic systems were simultaneously activated, but to varying degrees under different circumstances, and that the final response represented a net summation of the activity of the two systems.

Wener's patient was subsequently subjected to bilateral vagotomy in an effort to save the proximal half of the colon, since it had begun to show early changes of disease (hyperemia and edema but no ulceration).[13] Immediately postoperatively there was temporary pallor and hypomotility of the colon, which slowly returned to almost the preoperative state in six weeks. The character of the response to naturally occurring and experimentally induced emotional states was similar to that obtained preoperatively but the degree of vascular reaction of the mucosa was reduced. The authors, however, acknowledge that the difference might be due to the fact that the patient "was more accustomed" to the experimentors and the experiments. In the light of Grace's report that vagotomy did not alter the function and behavior of the colon in their one subject who had a severe exacerbation a few days after the vagotomy, our experience with a patient who thought she had a vagotomy is of interest.

A twenty-five year old woman was suffering her second severe bout of ulcerative colitis. After a progressive downhill course over five months a vagotomy was attempted as a desperate measure. Because of the unexpected finding of an enlarged, friable spleen, the procedure could not be carried out and the incision was closed with nothing having been done. Within ten days she was out of bed and feeling fine. She remained free of any symptoms of ulcerative colitis for one and one-half years. The patient remains under the impression that vagotomy was done and until the relapse, which was mild and short-lived, was unstinting in her praise of vagotomy as a cure.

Dennis et al. observed tense engorgement of the mucosa, excessive secretion of watery fluid and the appearance of petechiae and superficial hemorrhages when he brought up certain emotionally charged topics while sigmoidoscoping two patients with ulcerative colitis.[14] Four to five days after vagotomy no corresponding responses were noted.

Posey and Bargen studied motor function with tandem balloons in two patients with chronic ulcerative colitis who had stomas in areas of active disease in the descending colon. Both patients showed complete intersegmental coordination, with no normal intersegmental blocking mechanism apparent. There was virtually an incessant discharge of material from these stomas. There was abnormal, excessive and constant hypermotility. No intervals of quiet or even relative quiet appeared on the records.

Spriggs et al. studied motility in ten patients with ulcerative colitis by means of a tandem balloon system inserted through a proctoscope.[16] All had active involvement of the rectosigmoid. The motility records were analyzed quantitatively. They found that the tracings obtained from the pelvic colon of patients with ulcerative colitis showed less motility than those from normal persons; type II waves, which typified records from normal persons, occurred less frequently, and type IV propulsive waves, which were absent in the records for normal persons, were characteristic of the records from patients with ulcerative colitis. No differences were noted in either group before and after the ingestion of food. The authors believe that type IV waves represent a mass synchronous contraction of a length of colon and that the type II waves represent haustral movement. Radiologic haustra are lost in severe ulcerative colitis.

Kern et al. also studied sigmoid motility by single or tandem balloons in forty-five patients with ulcerative colitis.[17] In twenty-one experiments on sixteen patients there was no phasic

activity of the colon, a phenomenon never observed by them in patients without ulcerative colitis; seventeen patients showed reduced activity; seventeen had activity within the normal range; and four displayed exaggerated phasic activity. In nine of the seventy-two tracings a large propulsive wave (type IV) was found. The duration of illness and x-ray appearance could not be correlated with the type of sigmoid activity. There was, however, a correlation between the degree of diarrhea and the character of the sigmoid motility tracing. In general, tracings with little or no phasic activity were found in those patients with the most severe diarrhea. The type IV waves also occurred in patients with marked diarrhea. Those patients in whom the disease was still active, as shown by proctoscopy, but who did not have diarrhea, had a normal amount and type of activity. The patients who had hyperactive tracings were actually constipated. This association was confirmed in five patients during different stages of clinical activity of the disease. Disturbing personal interviews productive of emotional conflict resulted in an increase in amplitude and frequency of contractions in six patients with reduced spontaneous activity. Similar studies made in seven patients with right-sided colitis without involvement of the sigmoid revealed normal patterns of sigmoid activity.

The authors believed that the absence of wave-like activity in the sigmoid may represent sustained contraction and that the type IV waves represent the progression caudad of waves of mass peristalsis initiated in the proximal portion of the colon. Similar reduction in phasic activity in the sigmoid has been observed in experimentally induced diarrhea in dogs[18] and was observed fluoroscopically by Hurst in man during mass peristalsis.[19] Kern et al. have also been able to reproduce it temporarily by methacholine and acetycholine in normal subjects.[20] They therefore suggest that the colon of the patient with ulcerative colitis is subject to constant autonomic, probably parasympathetic stimulation, and that this functional state is maintained for long periods, even in the absence of the usual stimuli for mass peristalsis.

Comments. Direct observations of the colon are so far restricted to only three patients. In all three the right colon only was available for study, while the main site of the disease was in the left colon. On only a few occasions did the observed changes progress to a state consistent with active ulcerative colitis, and then only the earliest changes were noted (petechiae). The sequence of events is important. Grace et al. describe a synchronous change in motor, secretory and vascular function. Winklestein, observing one of the same patients, claimed that petechial hemorrhages were noted during extreme vascular engorgement in the absence of any contractions of the bowel. In Wener's case, when the mucosa was engorged and edematous, contractions then led to scattered petechiae and easy bleeding. Clearly the data are too limited to permit any conclusions but they make doubtful the concept that vascular changes are the result of spasm of colonic musculature. In any event, the value of direct observation should not be overemphasized.

The studies of motor function also provide evidence that vascular and motor behavior may be independent since Kern found normal patterns of motor activity in the sigmoid of patients who still showed active disease by proctoscopic examination but who were having no diarrhea. Sustained contraction, as evidenced by absence of phasic activity, and mass synchronous waves correlated with constipation, were evidently independent of the vascular status. Code's generalization that "the majority of patients who have had this disease for a period of years display a reduction in the total amount of activity in their colons, accompanied by a shift from mixing and absorption-promotion types of motility toward a propulsive excretory type of activity" is undoubtedly correct[21] but it should not be interpreted to mean that an increase in propulsive activity is necessarily an essential component in the development of ulcerative colitis. Were this so we should expect diarrhea regularly to be an early manifestation, which it is not. To clarify these points not only are more detailed serial observations of motor function during the complete course of ulcerative colitis necessary but also corresponding studies of other types of dysentery.

In attempting to relate the various aspects of colonic function, the normal differences in behavior of different parts of the colon and the special circumstances of the experimental observations must be taken into account. The direct observations were all of the right colon while the balloon studies were of descending colon and rectosigmoid. In one of Grace's cases a fairly sizable segment of ascending colon had prolapsed through a cecostomy. In

the second case the prolapsed segment was small so that direct observations on motor activity were difficult. In Wener's case only the cuff of the transverse colostomy was available for observation and motor activity was recorded by means of an inflated latex balloon inserted 6 to 8 inches through the colostomy. The cecostomy patients also had ileostomies, which means there was an interruption of the continuity between small and large intestines. Posey observed that there never was propagation of peristalsis across the gap of a double-barreled stoma, that each limb appeared to be permanently independent of the other once bowel was transected.[15] Caution is therefore required in transposing the results of these observations to the status of the patient with ulcerative colitis whose colon is not surgically interrupted. The character of the motor activity also requires careful analysis. Grace's patients showed both an increase in rhythmic contractile activity, probably segmental, and a generalized constriction and shortening of the exposed segments of right colon, the latter suggesting mass type of activity. In addition they speak of increased reactivity to mechanical stimulation, although they do not clearly indicate what kind of motor response occurred. Wener's studies are incomplete in clarifying the nature of the motor activity, although they are quite convincing in indicating a dissociation between the motor and vascular components. Using a single balloon, unspecified in size, it is not possible to distinguish between segmental contractions and mass waves, even on occasions when the balloon is expelled by a "rush of peristalsis." Whether or not there were sustained tonus changes, such as those noted by Grace in the left colon, also is not clear from their protocols.

The crucial question as to whether the colon is responding as a unit in a general exaggeration of the excretory pattern or whether local areas are "irritated" and set in motion colonic activity remains unanswered by these studies.

V. LYSOZYME

Since this enzyme has been proposed as a factor in the pathogenesis of ulcerative colitis, some comments as to present knowledge of its status is indicated. No conclusive proof or disproof of a damaging action by lysozyme on the colonic mucosa has been given but there is considerable evidence that the high concentration of lysozyme demonstrated in the stools of patients with ulcerative colitis is a concomitant of the inflammatory response, not a cause. Thus Hiatt et al. found the invading granulocyte to be the primary source of lysozyme in the stools of patients with ulcerative colitis.[34] They showed that when the leukocytes were separated from the mucus, the mucus fraction was devoid of lysozyme unless considerable autolysis of the white cells had occurred. They also proved that granulocytes did not absorb lysozyme from the surrounding media. These studies, plus the earlier studies of Meadows,[35] Janowitz[36] and Glass[37] demonstrating that lysozyme has no mucolytic action against colonic mucus, make it extremely doubtful that lysozyme plays any role in the genesis of ulcerative colitis.

Grace found high values of lysozyme in the mucosal secretions and stools of patients with ulcerative colitis immediately preceding episodes of exacerbation of symptoms with bleeding.[10] Since the material was not examined for white cells this source cannot be definitely implicated. However, it could mean that white cells migrate into the secretions very early in the pathologic process, a phenomenon well worth careful scrutiny, especially in light of Warren and Sommer's suggestion of the possibility that some substance is secreted into the crypts.[5]

VI. EXPERIMENTAL PRODUCTION OF COLITIS IN ANIMALS: RELATION TO THE DISEASE IN HUMANS

The experimental production of ulcers in the colon was reviewed by Ginsberg and Ivy in 1946.[22] We will concern ourselves only with procedures which lead to lesions identical with or closely resembling ulcerative colitis in humans.

A group of workers from the Mayo Clinic and from the Pasteur Institute claimed that the injection of Bargen's diplococcus or other organisms in dextrose-brain broth may produce an acute hemorrhage and ulcerative lesion of the colon in rabbits and dogs. The pathologic changes were largely restricted to the colon, none being noted, or only rarely, in the upper gastroenteric tract. The reader is referred to the monograph by Bargen[2] and to the reviews by Ginsberg and Ivy[22] and Monaghan[7] for discussion of this work. Even though these studies do not prove the specificity of Bargen's organism in the pathogenesis of ulcerative colitis, the fact that a lesion closely resembling ulcerative colitis could be produced by these technics remains of interest. Unfortunately the controversy about this work has mainly concerned the specificity of the organism and it has become fashionable to re-

gard it as of little importance since there is general agreement that Bargen's organism is a normal inhabitant of bowel.[7,23] But this ignores the fact that although a number of investigators have been unable to reproduce these results, Bargen's group and five independent investigators have produced colonic lesions in experimental animals by this technic, a fact which justifies careful re-examination in the light of new knowledge.

It has been suggested that some or several factors might operate to alter the susceptibility of the colon to organisms normally present or to alter the population of organisms.[22,24] The development of a colitis during the use of aureomycin and chloramphenicol[25] and with folic acid analogues,[26] while of a type not pathologically identical with ulcerative colitis, may have some bearing on this problem.

Lium prepared colonic explants in dogs and, having demonstrated that the bowel could be maintained in normal condition when protected by dressings, he studied the effect of induced muscular spasm on the explant.[27,28] When the mucosa of the graft was pinched with a hemostat there resulted a severe spastic contraction of such marked degree that the graft felt "like a mass of cartilage." At times the graft relaxed slightly, only to resume its contractions. Immediately after the pinching a large amount of mucus was poured out on the surface, and not only was there bleeding from the points damaged by the snap but petechial hemorrhages appeared beneath the mucosal surface in the areas that had not been pinched. At times these areas bled into the mucous coat on the surface of the graft. These contractions lasted for one-half hour and then gradually receded. After four days the traumatized areas were completely healed.

In another series of experiments 0.5 cc. of 1:4000 prostigmine was given intravenously and immediately thereafter at five-minute intervals for forty minutes 0.2 cc. of 1 per cent solution of an acetylcholine salt was applied to the surface of the graft. In all six animals the grafts contracted violently immediately after the acetylcholine was applied and the color changed to a pale yellow. There was alternate contraction and relaxation but the graft did not relax to its original size. On relaxing it became pink. Within fifteen minutes every explant showed petechial hemorrhages which gradually increased in size and coalesced. In the dogs which showed a maximum response the surface at the end of an hour appeared like a piece of raw beef. There was a rich secretion of thick mucus during the early part of the experiment but this gradually changed to a thin, watery secretion. After forty-eight hours the grafts again looked normal. Microscopic examination at the end of the period of application showed distention of the subepithelial vessels, with rupture in some places and bleeding on to the surface of the mucosa; absence of mucus from the cells, and loss of surface continuity of the epithelial cells in a number of places, with formation of small erosions.

Intravenous injection of Shiga toxin produced vomiting, diarrhea and bloody stools, and fifteen seconds after the injection a powerful, spastic contraction in both circular and longitudinal fibers of the grafts. Associated with the spasm was edema and induration, petechial hemorrhages and oozing of fresh blood from the graft. The following day the graft appeared hyperemic and edematous and showed punctate ulcers which bled into the mucus on the dressing. There was no reaction when the toxin was applied directly to the mucosa.

From these observations Lium is led to the belief that spasm is a prime factor in the causation of ulcerative colitis. He finds clinical support for this point of view in the fact that the disease usually begins in the rectosigmoid, which is the location of the strongest muscles of the colon. His report of the linear distribution of ulcers along taenial bands has already been commented on. Lium suggests that local spasm of the smooth muscle will shut off the blood supply to the surface layers for the period of the contraction.[29] He states this can be observed directly in the exposed colon during the act of the defecation reflex, the blood vessels being compressed to a sharp point along the mesenteric border, no blood passing through them for the duration of the severe contraction. In observations of the defecation reflex in decerebrate cats with plexiglass windows sutured into the abdominal wall Lium describes the colon as a "solid, blanched cord, with a complete shut-off of the blood to the mucosa" at the end of defecation. He concludes that the postulated severe spasm in ulcerative colitis is an exaggeration of the normal reflex of evacuation.[29]

Wener, Hoff and Simon reported they could produce ulcerative colitis in dogs by the prolonged administration of mecholyl.[30] The patho-

logic changes ranged from severe hyperemia, interstitial mucosal hemorrhages with superficial necrosis, erosions, focal areas of acute colitis and acute or subacute ulcerations. The outstanding feature of these experiments was the marked bloody diarrhea which came on soon after each injection of mecholyl. The stools were well formed at first and of normal color and consistency, but within ten to fifteen minutes subsequent bowel movements were looser and blood-tinged, and eventually became watery, mucoid and bloody. The vomitus was frequently blood-tinged and occasionally massive hematemesis occurred. Bloody diarrhea usually disappeared in two to three hours and within twelve to twenty-four hours the stools became formed but tarry. Many animals died in shock and with massive hemorrhage. Generalized parasympathetic responses accompanied every injection. These investigators believed that the vasodilator action of mecholyl on the blood vessels was the main factor in the pathogenesis of these lesions. They suggested the following sequence of events: The marked vascular hyperemia and engorgement gave rise to stasis, increased capillary permeability and tissue anoxia, which resulted in extravasation of red blood cells into the mucosa, which was followed by superficial necrosis of the mucous membrane, erosions, and finally led to the formation of true ulcers. They believe the vigorous contractions of the large bowel upon an engorged, friable mucous membrane may have been an additional factor.

Comment. It is doubtful that the disorder known in humans as ulcerative colitis has been produced in animals. Actually, the disputed experiments of Bargen come closest to achieving this goal and if for no other reason this justifies their careful repetition. The importance of Wener, Hoff and Simon's claim can easily be overestimated because they reserve the description of the effects of mecholyl generally and on the rest of the gastroenteric tract for another paper.[31] Thus although they clearly refer to this paper by saying that "the generalized parasympathetic responses which accompany every injection of mecholyl have already been described," the reader who has not read the first paper can easily fail to appreciate the extent of the changes elsewhere. Of the forty-eight dogs used all but five died of general effects of the drug. Within one to two minutes after the mecholyl was given sweating, panting, salivation, lachrymation, retching, excessive vomiting, urination and defecation were noted. Some of the animals went immediately into shock and died within an hour. Many died from massive hemorrhage. Several had bilateral adrenal hemorrhages. Hemorrhagic lesions and erosions were found throughout the stomach and small intestines as well as colon. These results are similar to those resulting from continuous stimulation of the vagus in dogs[32] and to the effects of removal of the celiac and superior mesenteric ganglia.[33] The experiments of Wener et al. have some bearing on the problem but their claim to have produced ulcerative colitis experimentally cannot be accepted. Their studies, however, do indicate that parasympathetic overactivity as induced by the drug mecholyl can lead to changes similar to those seen in the human disease. That this can be brought about in a purely local manner is shown by Lium's experiments with colonic explants.[28] However, Lium also showed that Shiga toxin intravenously, and rubbing and pinching produce similar changes. We are thus observing a category of colonic responses which are probably brought about in a number of ways, including parasympathetic activity. When such changes are observed, as in the colon of patients with ulcerative colitis, it is not clear as to whether they were initiated centrally (i.e., via the sacral outflow) or through circulating substances (as with Shiga toxin) or locally. In the opinion of the writer, the work of neither Wener nor Lium provides convincing evidence for overactivity of the parasympathetic division as a primary factor in the pathogenesis of ulcerative colitis.

Lium's concept of the primacy of muscular spasm in the genesis of the mucosal lesion is impressively demonstrated in his experiments but is not well borne out by the direct observations in the stoma patients. In none of the latter is there any report of an ischemic contraction. Indeed, more often mentioned is that hypermotility and engorgement occurred together,[10] while in other instances petechial lesions developed in the absence of hypermotility.[11,12] However, all these observations were on the less muscular proximal colon. The ischemic factor might be more important in the rectum and sigmoid. Lium's data are important in indicating that when muscle spasm is severe enough to produce ischemia, mucosal damage more quickly ensues. This may well bear on the predilection for the disease to develop earliest in the rectosigmoid and may be of primary importance in

the small group of patients whose illness begins with obstinate constipation. Lium would relate this to an exaggeration of the normal reflex of evacuation. His description of this process as observed in decerebrate cats with plexiglass windows sutured in the abdominal wall is of interest: "The nervi eregeus were threaded onto electrodes and stimulated. The first event in the act of defecation is the formation of a contraction ring. All the matter below the ring is evacuated. The next step is a contraction of the longitudinal muscle fibers with a consequent shortening of the rectum and sigmoid. The contraction ring then spreads downward rapidly; the sphincters relax; and involuntary straining takes place with evacuation of the fecal mass. At the end of defecation, the colon is a solid blanched cord, with a complete shut-off of the blood to the mucosa. This extreme spasm is only temporary."[29] Repeated and long-sustained contractions of this sort could, in Lium's opinion, lead to the changes of ulcerative colitis. The symptomatology of the acute constipation cases certainly suggests such a mechanism but only a small proportion of cases begin in this manner. The story of such patients often sounds like a fruitless attempt to pass a non-existent fecal mass. Even in such cases, however, we are left without information as to whether such an evacuation response is initiated by mucosal irritation or a central stimulus acting through the sacral outflow. That the majority of cases do not begin in this fashion indicates either that this is not the usual mechanism or that it is not reflected in the symptomatology of most patients. The motor studies, which we have already discussed, contribute little to the resolution of this question since they were all carried out in patients in whom the disease was already well established. The observation of patients with active disease but normal motor patterns may be regarded as evidence against the spasm hypothesis[17] but this is hardly conclusive since it may be argued that the factors which initiate the process and the factors which sustain the process may not be the same.

Wener's assertion that the colonic changes induced by mecholyl in dogs are primarily vascular and that muscular contractions acted as an additional factor is an inference and is not based on any observations reported in his paper.[30] Kern and Almy[20] and Code[21] observed that parasympatheticomimetic agents stimulate motility of large bowel and do so by altering the character of the motility from a predominantly non-propulsive to a predominantly propulsive action. In the absence of direct observations primacy cannot be ascribed to either process and certainly no final conclusions can be drawn with respect to ulcerative colitis.

VII. STUDIES OF DIARRHEA AND CONSTIPATION IN PATIENTS WITHOUT ULCERATIVE COLITIS

Grace[10] and Almy[38] report that diarrhea is associated with vigorous rhythmic contractions of the right side of the colon while the region of the sigmoid colon undergoes longitudinal contraction with shortening but displays no rhythmic contractions of the circular muscle. The pattern of motility in the sigmoid colon during constipation is less consistent, sometimes showing hypomotility and sometimes non-propulsive activity of circular muscle.

Comment. Motility studies on patients with diarrhea and constipation reveal a gradation between propulsive and non-propulsive and less non-propulsive activity than the others, but only when they are having active diarrhea. Such studies, therefore, reveal only that motor behavior is consistent with the degree of bowel activity and do not give any information as to the initiating mechanisms of diarrhea. Again this is an expression of the fact that the bowel can only respond in a manner determined by its morphology and functional organization. More studies are needed of patients with diarrhea due to specific enteritides and due to chemical agents.

VIII. ALLERGY

Gray and Walzer[57,58] have succeeded in passively sensitizing the mucous membrane of rectum, colon and ileum in humans and demonstrating the nature of the hypersensitivity reaction. This they achieved by injecting a human reagin-bearing serum (peanut) into the mucous membrane of rectum in thirty-eight patients and of ileum and colon, respectively, of two patients with exposed bowel segments and then observing one or two days later the response to oral administration or local application. The description of the allergic response is important. In the rectum the onset of the local reaction is heralded by pruritus, burning, sense of fullness and desire to defecate when close to the anus, and with just fullness when higher. Edema and pallor were the first observed changes. The edema reached its peak in five minutes and

then hyperemia developed and grew in intensity for ten minutes, the mucous membrane becoming deeply congested. There was increased mucus secretion and the total reaction lasted about an hour. The thirty-eight patients who were thus studied represented a variety of diagnostic categories but only one was listed as having "ulcerative proctosigmoiditis." There is no mention in the paper whether the reaction was any different in this patient.

In the two observations on ileum and colon studied by the same technic pallor of the mucous membrane and an increase in mucus secretion were usually the first objective signs. Edema reached its height in fifteen to twenty minutes and persisted for one hour or more. Pallor was gradually replaced by hyperemia.

Atkinson, in a very brief discussion of the first paper, claimed that he produced similar phenomena in dogs in exteriorized segments of the colon by actively sensitizing the dogs to various foreign proteins. In some of the dogs ulcers developed but they healed in spite of repeated injections.

Rubin described a syndrome in three to five week old infants characterized by colic and bleeding which he believed to be an allergic sensitivity to cow's milk.[59] No observations are available as to the exact site of the bleeding but the presence of bright red blood and mucus in the stool suggests that it was colonic. Three of the four infants developed eczema at four months.

Comment. The demonstration of passive sensitization of colonic mucous membrane is important but its relation to ulcerative colitis is less clear. The prominence of pallor in the local response may be contrasted with what has been described in direct observation of the colon of patients with ulcerative colitis. No mention is made of the development of petechiae in the hyperemic phase of the response yet, if Rubin's cases are pertinent, a hemorrhagic response is certainly possible. It is entirely possible that these differences are related to minor circumstances of the experiment and are not of fundamental importance.

PSYCHOSOMATIC HYPOTHESES

We are now in position to examine the adequacy of various psychosomatic hypotheses. The formulation of such hypotheses has always presented great difficulty because they require relating data obtained in two different frames of reference. Depending on the background of the investigator, there has been great unevenness in the precision of observations as well as of concepts. In some instances psychologic data have been collected with great care but the observation and even identification of the somatic processes has been crude and inaccurate. In other instances the reverse has been the case, precise physiologic data being related to inadequate, impressionistic psychologic data. In both situations the resulting formulations have inevitably tended to oversimplify or distort either the psychologic or the somatic aspects of the disorder, or both.

To avoid any possible misapprehension we wish to re-emphasize our basic premise, that psychologic determinants are intimately involved, in some as yet unexplained way, in the pathogenesis of ulcerative colitis. That psychologic processes are involved in all diseases goes without saying but clinical observation leads one to be more impressed with the role of psychologic factors in the pathogenesis of this disease than, for example, in measles.* Therefore the discussion that follows is not to be construed as an attempt to refute this fundamental premise. Rather it will be an attempt to evaluate hypotheses in terms of how adequately they correspond with what we have been able to learn about the nature of the somatic process. The psychologic data will be analyzed in another paper.[1a]

To achieve this we will first list the data which we believe must be taken into account in any hypothesis: (1) The disease may begin at any age, from birth to old age, but most cases begin in adolescence and early adulthood (fifteen to thirty-five years of age). (2) The most common first manifestation is bleeding. Diarrhea is *not* the usual mode of onset. (3) A significant number of cases begin with severe constipation, bleeding beginning soon after. (4) The nature of the bowel movements, whether diarrheal, formed or constipated, is dependent on the extent and severity of the pathologic process. When restricted to rectum or rectosigmoid, formed stools or constipation is usual, whereas with more extensive involvement or right-sided involvement, diarrhea is the rule. (5) A high proportion of patients have a premorbid history of constipation; a smaller number have had brief episodes of diarrhea. (6) The

* For a fuller discussion of a unified concept of health and disease the reader is referred to another paper.[39]

same process may develop after ileostomy and colectomy in an ileum which was demonstrated to be normal at the time of surgical intervention. (7) Active disease may persist or reappear in bowel by-passed by ileostomy. (8) The disease most commonly begins in the left half of the colon but may begin in any segment and may be segmental or patchy. (9) Pathologic and sigmoidoscopic observation indicates that the main sites of early lesions are the submucosa and mucosa. (10) Direct observation of surgically exposed colon in a limited number of patients indicates the development of early changes of ulcerative colitis to be consistently preceded by hyperemia and inconsistently by hypermotility and hypersecretion. (11) There is a shift toward propulsive type of motor activity and away from mixing and absorption-promoting activity in patients with diarrhea whereas patients who have no diarrhea but still have active disease (as revealed sigmoidoscopically) may have normal motor patterns. (12) While the lysozyme titer rises during active ulcerative colitis the evidence indicates that this comes from the granulocytes and from granulation tissue and that this enzyme does not damage colonic mucosa, at least in the concentrations in which it is ordinarily found. (13) While parasympatheticomimetic substances produce acute changes which resemble those seen in early ulcerative colitis, it has not been possible as yet to reproduce the disease in experimental animals by any such technic. (14) No specific microorganism has been implicated in the pathogenesis of the disease but until Bargen's experiments are repeated the possibility of an altered reaction to organisms cannot be excluded. (15) Passive sensitization of the colonic mucosa has been produced but the hypersensitivity reaction differs in some respects from the changes observed in patients with ulcerative colitis.

In the discussion that follows the points above will be referred to by numbers when cited as evidence:

Sullivan.[40] "Emotion whips the liquid contents of the small intestine down into the colon. In these particular individuals the enzymes in this liquid intestinal content may be of higher digestive power than the normal or the natural protective powers of the mucosa of the colon may be lowered. At any rate, surface digestion of the mucosa of the colon occurs, bacterial invasion is made easy and acute ulceration results. Because the emotional difficulty remains a chronic one, either because the situation cannot be solved or the patient is unable to face his problems, the hypermotility of the intestinal content persists and the constant irritation results in a chronic colitis."

Comment: Against this concept are points (2), (3), (4), (7) and (8). In addition, studies of proteolytic activity of the feces of patients with ulcerative colitis show wide variations and no consistent relationship with the activity of the disease.[41] Also in dogs diversion into the colon of bile, pancreatic juice and normal duodenal content does not result in any injury to the mucosa.[42,43]

Alexander.[44] The Chicago group have been impressed with the prevalence of those emotional factors which from early life are associated with the excremental and alimentary functions. "The first symptom of ulcerative colitis frequently appears when the patient is facing a life situation which requires some outstanding accomplishment for which the patient feels unprepared. The psychodynamic implication can be best understood on the basis of the infant's emotional evaluation of the excremental act, which signified giving up a cherished possession on the one hand and an accomplishment on the other. In persons with this type of emotional fixation, whenever the urge or necessity to give arises in later life or the realization of an ambition on some adult level is blocked by neurotic inhibitions, a regression to the anal form of giving or accomplishment may take place. It should be emphasized, however, that anal regression of this type is extremely common in all kinds of diarrhea and in psychoneurotics who do not display any somatic symptoms. Some specific local somatic factor may be responsible for the fact that in some patients anal regression produces ulceration in the bowel. It is quite probable that the specific factors will turn out to be not psychological, but the peculiarity of the physiological mechanisms initiated by the emotional stimuli."

Alexander then follows the suggestion of Portis concerning the physiological mechanisms.[45] Alexander writes: "Certain emotional conflict situations transmit a nervous excitation through the vegetative nervous system via the parasympathetic pathways to the colon. Sullivan assumed that the digestive power of the fast moving liquid content of the small intestines is higher than normal or the natural protective power of the mucous membrane of the colon

may be low. At any rate, surface digestion of the mucosa occurs and prepares the way for bacterial invasion. In this way acute ulceration results." He cites Portis' view that lysozyme "deprives the mucosa of its protective mucin," and that "the initial localization of the ulceration is always in that part of the large bowel which is under the nervous control of the sacropelvic portion of the parasympathetic system." Alexander believes this localization in early cases "confirms the psychiatric observation that the psychological stimuli involved pertain to the excremental act." He concludes his comments with the cautious statement, "The relative significance of inherent constitutional factors concerning the vulnerability of the mucosa of the colon—i.e., disturbed physiological mechanisms based on previous local disease of the colon—invasion by microorganisms, and specific emotional conflict situations cannot be evaluated at the present state of our knowledge."

Comment: Our observations, as well as those of many others, amply confirm Alexander's statement that psychologic material related to the psychodynamics of the excremental function is conspicuous in these patients. We will reserve for another paper a critical discussion of the bearing of such data on the pathogenesis of ulcerative colitis.[1a] Implicit in Alexander's concept, as it relates to the somatic process, is that it is intimately related to the process of elimination. The major focus is on diarrhea. Part of the formulation is a modification of that of Sullivan, which has already been discussed. The suggested role of lysozyme has not been borne out by more recent studies.[34–37] The statement that the disease "always" begins in the region of the sacropelvic outflow is not correct since it ignores right-sided colitis and the segmental types.[2,5] While admittedly such cases are much less common, it is not justified to use the more usual left-sided location as evidence for the "excremental" theory, especially since the psychologic data in right-sided colitis cases do not seem to be different. The fact that similar psychodynamic material is found in patients with regional enteritis also is ground for reservations.[46]

Of importance to Alexander's thesis would be the question whether in the initiation of the ulcerative colitis process the first behavior of the colon is an exaggeration of its excremental pattern. The evidence on this point is by no means clear. There is certainly good evidence that the colon does not necessarily respond at the outset with an integrated excretory pattern, for were that the case one would expect diarrhea invariably to be an early symptom. Indeed, the data suggest a dissociated or segmental response, both anatomically and physiologically. Anatomically, not only does the disease usually begin in one segment and later spread, but also it involves mucosa and submucosa preferentially. Physiologically, it has been observed that vascular, secretory and motor functions are not necessarily synchronous, or appropriately integrated for defecation. Indeed, among the patients with chronic bleeding and constipation there is reason to believe that the involved rectum or rectosigmoid is reflexly protected from the fecal stream by inhibition of propulsive activity of proximal segments. We have found no evidence that the constipation in such cases had primary psychologic meaning.

Thus although there are cases in which the sequence of events is compatible with Alexander's concept, in the majority of cases the beginnings of the disease are not characterized by an exaggeration of excretory pattern, even though the psychologic content is of such a character.

Szasz. In a series of papers which do not specifically deal with ulcerative colitis but rather with diarrhea and constipation, Szasz attempts to meet some of the aforementioned objections to Alexander's formulation.[47–50] He sees diarrhea as a consequence of an exaggerated gastrocolic reflex and believes it to result from a sudden decrease in vagal tonus to the stomach. He relates this to what he calls the "basic physiologic rhythm of the gastrointestinal tract," namely, that "in the infant (or in undomesticated animals) defecation occurs in a definite pattern: *The nursing infant experiences hunger, feeds, then defecates and falls asleep*" (author's italics).[47] He suggests that during hunger there is increased vagal activity and the large bowel is inhibited (through inhibition of the sacral parasympathetics or sympathetic stimulation), upon satisfaction of hunger there is decreased vagal activity and activation of the colon and rectum ensue. "Chronic (psychologic) stimuli of either type, i.e., stimuli which result in either chronic vagal activity or inhibition, may produce what may be regarded as exacerbations of the essentially normal pattern."[47] "Psychoanalytic studies of a variety of patients revealed that colonic and rectal hypo- and hyperactivity could be correlated most accurately with changes in the

patients' unconscious oral intaking tensions (drives)."[47] In the same paper he states that this hypothesis is in harmony with the clinical characteristics of ulcerative colitis.

Involved in this formulation is also Szasz's concept of "regressive innervation" which he defines as "an increased state of excitation of functionally specific (localized) parasympathetic pathway."[50] Since the parasympathetic division develops earlier than the sympathetic, Szasz believes that regressive innervation represents a return to the oldest, most archaic method of adaptation to stress. He considers ulcerative colitis a disease of "regressive innervation." He interprets the data on lysozyme increase in ulcerative colitis as being the result of parasympathetic activity, thus one of the physiologic consequences of regressive innervation, and suggests that it is responsible for denudation of mucous membrane and ulceration.

Comment: Although from the purely psychologic point of view Szasz's concept is a contribution to the understanding of some varieties of diarrhea, it is not consistent with the observed facts in ulcerative colitis. All the criticisms of Alexander's formulation apply with equal validity to Szasz's. That the gastrocolic reflex is often more prominent in patients with ulcerative colitis is attested to by the frequent report by patients of cramps and bowel movements after eating, but this is also true in bacterial and amebic dysentery and diverticulitis. This may be a perpetuating factor but there is no evidence as yet that it is an initiating factor in ulcerative colitis.

The concept of parasympathetic activity as a "regressive innervation," as formulated by Ssasz, is open to serious criticism, which cannot be fully considered here. Behavior may be regressive but it is doubtful that the term is properly applied to the function of the parasympathetic system which, in my opinion, mediates appropriate (i.e., a normal bowel movement) as well as inappropriate responses. Further, the sharp distinction between the functions of the sympathetic and parasympathetic divisions is not consistent with modern knowledge of the autonomic nervous system. The evidence that the so-called "basic physiologic rhythm of the gastrointestinal tract" is an expression of the dominance of the parasympathetic division is only weakly supported by evidence.[48,50] But the most damaging fact is that vagotomy in the ulcerative colitis patient does not result in "a sensitive and irritable bowel," functioning "like the corresponding organ of the suckling baby."[50] Indeed, the procedure is so innocuous that it has been recommended as a form of therapy.[14] The patients of Grace[10] and of Wener[18] showed no change in colon function or gastrocolic reflex after vagotomy, indicating that at least the right half of the colon does not become overactive. In addition, vagotomy has no effect on colonic motility in dogs.[51] One might question that the more frequent bowel movements of the infant means the bowel is more "sensitive and irritable" and that this is necessarily evidence of the parasympathetic activity.

The crux of the matter is that even if the mechanisms suggested by Szasz were verified they would still account only for diarrhea and not for the characteristic changes of ulcerative colitis. So far there is no evidence that the kind of mucosal-submucosal reaction typical of ulcerative colitis has its counterpart in infancy, although there remains the possibility that such patterns may exist or may be established at least among the children who in later life develop ulcerative colitis. Should this prove to be the case, there is still no reason to assume that it represents a "regressive innervation" via the parasympathetics. Some type of cellular or tissue change, such as might follow an infection, for example, is equally conceivable. This will be discussed in more detail in the conclusion of the paper.

Grace, Wolf and Wolff.[10] These investigators see ulcerative colitis as part of an ejection-riddance pattern involving the large bowel. "A subject confronted by overwhelming environmental demands may elaborate a pattern of ejection. Thus, a person who has taken on 'more than he can handle' or feels inadequate to the demands of his life situation, or a thwarted and passive person filled with hatred, defiance, contempt, or the unconscious aim to eject a threatening or overwhelming situation may have diarrhea. However, the riddance pattern being integrated through unconscious processes, the subject exhibiting violent diarrhea may be calm, sweet mannered, and seem serene." They suggest that the choice of colon as the particular organ to participate in the particular defensive pattern in any individual is "stockbound," that some people are for constitutional reasons "colon-reactors," others "nose-reactors," "skin-reactors," etc.

According to this view, in a setting of conflicts

with "anxiety and resentment," the colon is hypermotile, hyperemic, engorged, fragile and susceptible to injury so that it is possible that otherwise harmless agents may bring about further injury, bleeding or ulceration. They suggest that such agents may be constituents of normal stool, digestive enzymes or indigenous bacterial flora. Thus they see a summation of factors in the pathogenesis of ulcerative colitis.

Comment: The concept of a basic "riddance" pattern appears amply supported by biologic facts and the colon has this as its major function. What is much less evident, however, is whether the primary reaction of the colon in ulcerative colitis is its "riddance" pattern. As with the previous formulations, this concept emphasizes diarrhea as the basic phenomenon whereas the data here cited open this to serious question. Again the points raised in criticism of this aspect of other concepts also apply here. However, Grace states that in adults noxious symbols seldom evoke a full-blown integrated protective response but more often a fragmented one. "The terminal portion of the large bowel may be involved in a rejection pattern, whereas the ileum and the jejunum may be functioning in an average way." He cites examples from other systems where only parts of protective patterns are activated. This is an important modification which is in accord with the fact that the colon does not behave as an integrated excretory unit in this disease. Regrettably, however, the authors do not provide any details as to how they see this fragmentation in ulcerative colitis, what functional or anatomic divisions or parts thereof are involved and in what order. Although they do not explicitly so state, in reference to the colon, the riddance-ejection pattern could involve trans-mucosal processes (hyperemia, transudation and increased secretion) as well as defecation processes. Available data strongly suggest the former as more likely to be primary in ulcerative colitis. However, there is no answer yet as to whether a noxious symbol can provoke such a "riddance" response through the lining of the colon or whether nonspecific processes associated with an affect, but having no protective or physiologic significance, are responsible. This will be discussed further in the paper on psychologic processes.[1a]

The suggestion that the "dominant protective reaction" is stockbound is not easily subject to examination. Bargen found that seventeen of 900 patients belonged to families in which two or more members had the disease (1.9 per cent) but these seventeen came from seven families.[2] Two of our patients had siblings with the disease (6 per cent). There is a high incidence of bowel disturbance antedating the onset of the colitis. The matter of genetic or constitutional factors is important but what they are and how they operate remains completely unknown.

The description of the hypermotile, hyperemic, engorged, fragile bowel is based on direct observation. It remains unsettled how the vascular and motor changes are related but there can be no question of the prominence of the former. While there may be considerable disagreement as to the interpretation of the psychologic data, there can be no argument that changes in psychologic status were associated with changes in the behavior of the bowel. Until more cases are studied and more refined technics are applied, the descriptions of those who have made this kind of observation must be the starting point for any consideration of the nature of the somatic process.[10–13]

Groen.[52] This writer follows the concept of Lium[27,28] and suggests that chronic anxiety gives rise to prolonged spasm of the muscles of the colon, ischemia of mucosa, leading to small necrotic lesions of the epithelium from which, on relaxation of the spasm, bleeding will occur. But the spasm does not subside completely so that the epithelium is insufficiently supplied with blood and healing is interfered with.

Comment: The merits of Lium's concepts have already been discussed. The direct observations of the development of the colitic process in the stoma patients provides the main evidence against this mechanism as a primary one, although there is good reason to believe that muscle spasm may be important in determining the location of ulcers.

Sperling.[53] This author's formulations are largely in psychologic terms. Obviously impressed with the bleeding in this disease, she speaks of unconscious rage with an irresistible urge for immediate discharge, leading to "the destruction and elimination of the object through the mucosa of the colon (bleeding)." The latter she regards as the specific mechanism in ulcerative colitis. "The faeces and blood (in severe attacks only blood and mucus) represent the devaluated and dangerous objects. In all cases with much bleeding, observed and analyzed, it appeared as if the quantity of blood was directly proportional to the intensity of uncon-

scious rage (oral and anal sadism) present at the time." She regards ulcerative colitis as an organ neurosis with pregenital conversion symptoms. The choice of the organ is determined by oral and anal fixations, the colon being the eliminatory organ. The anorexia, vomiting, abdominal pain, diarrhea and bleeding represent expressions of and defenses against aggressive incorporation of the frustrating object.

Comment: Sperling's formulation is notable in that it is the only one which is consistent with the pathology and clinical symptomatology of the disease. She recognizes the importance of the bleeding and the transmucosal processes. Yet it is not so much an explanation of the somatic process as a description of it in psychologic terms. It remains to be proven whether conversion mechanisms can involve such functional divisions as the mucosal surface of the colon, and to what degree the psychologic data of Sperling and others, including this author's, represents a secondary psychic elaboration of the meaning of the physical processes. Evidence on this point will be further considered elsewhere.[1a]

Daniels.[54,55] In his earlier paper Daniels speculates about the role of the parasympathetic nervous system in the genesis of the disorder and cites Sullivan's hypothesis.[54] Later he writes as follows: "The reasons for the capacity of the colon to respond as it does to a critical increase in anxiety (fear) cannot be discussed purely in psychological terms. They must be sought as well in the physiological and biochemical matrix of the individual and his gastrointestinal tract. There is the fascinating possibility that the same chemical and neurophysiological defects that interfere with the integrative activity of the cerebral cortex and subcortical centers reflected in the disintegrative psychological responses also produce the disintegrative behavior of the peripheral organ. Whether or not these defects center upon the cholinergic mechanisms of excitation or involve other still unknown neurohumoral factors, remains, of course, a key problem.

"The psychoanalytic investigation of adults cannot resolve the enigma of 'organ selection.' Although it is entirely possible that infantile fears such as those described and 'infantile conditioning' somehow create local somatic sensitivity to future tension, it cannot be proven by psychological studies alone."

Comment: Since Karush and Daniels do not specify what they consider the somatic processes to be, no comment is required other than a general agreement with their general attitude as to how the problem has to be approached. In this regard they stand in sharp contrast to Sperling.

CONCLUDING REMARKS

All the work summarized in this review has contributed significantly to our understanding of ulcerative colitis. However, it is clear that none of the psychosomatic hypotheses so far advanced has fulfilled the requirements both of correctly identifying the somatic processes and of indicating how psychic processes are related to the somatic. The jump from the psychic phenomena to the physical phenomena at the end organ is conceptually the most difficult and it is impossible to accomplish without a clear understanding of what can only be expressed in psychologic terms and what can only be expressed in physical terms.

What concept of the somatic process is most consistent with the material summarized in this review? In my opinion these data suggest that ulcerative colitis is a disorder involving primarily the mucosa and/or submucosa of the bowel, including at times the small bowel. Prominently implicated is the vascular system, so that hyperemia and hemorrhage are identifying features of the disease. The process characteristically does not involve all parts of the bowel with equal intensity and while the sigmoid is the area most commonly affected any part of the colon and ileum may be involved. The character of the bowel movements in any individual case is determined by the location, severity and extent of the colitic process. In other words, the bowel appears to be responding to local areas of surface irritation rather than as part of an integrated excretory act. The behavior of surgically by-passed bowel is also consistent with this.

From both the clinical and pathologic points of view ulcerative colitis more closely resembles the dysenteries of bacterial origin than it does mucous colitis. Indeed some pathologists believe it difficult, if not impossible, to distinguish the early stages of ulcerative colitis and bacillary dysentery. While attempts to establish an infectious origin have proven unsuccessful, the similarity to disorders of such origin is of significance. It suggests that we are dealing with a process in which the bowel surface is responding as if to a noxious agent of microscopic or molec-

ular size. This is in contrast to the response of the bowel as a whole to a bolus-sized agent, its usual excremental behavior. This conclusion is not essentially different from what has been generally suspected about ulcerative colitis since it was first described. Present knowledge does not permit any more precise identification than this. Included among the possible factors to be reckoned with are (1) reactions to known or unknown bacterial or viral agents and/or their products, (2) allergic phenomena, (3) Shwartzman type reaction, (4) reactions of the type associated with the collagen disorders,[9] (5) blood-borne chemical substances, (6) genetic or constitutional abnormalities of this level of tissue response or organization, especially as it involves the circulatory reaction, (7) metabolic or enzymic abnormalities. Are there other so-called psychosomatic disorders in which the physiologic derangements operate at a similar level of organization? One can immediately cite neurodermatitis, asthma and rheumatoid arthritis as three such conditions, and I believe there are others which have not been thought of as "psychosomatic" because the physiologic disturbance could not readily be formulated in terms consistent with psychologic theory. Skin and joint involvement are not uncommon complications in ulcerative colitis.

Some readers may be struck by the conspicuous absence of any reference to the parasympathetic nervous system in this conclusion. In all likelihood a wide variety of factors, including many already noted and mentioned in the text, result directly or indirectly in cholinergic responses. This is inherent in the functional organization of the bowel and is therefore a relatively non-specific phenomenon that tells nothing of the nature of the stimulus that provokes the cholinergic response. Concepts of "parasympathic overactivity" are thus meaningless.

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Seminars on Liver Disease

The Mechanism of Ascites*

A Physiologic Appraisal

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LIVER disease and the congestion of the hepatic and portal venous systems which are frequently accompanied by ascites have been regarded with interest for many years. Although studies of these conditions have been extensive, most of the reported observations on ascites formation have been descriptive rather than analytical. In recent years many clinicians and laboratory observers have re-examined the possible causes of ascites with the result that numerous studies are at hand. At this time these observations are largely scattered throughout the literature and are generally uncorrelated. Since the subject is of interest from the clinical and physiologic standpoints, it now seems justified to appraise the extensive data concerning ascites, as well as to focus attention on some of the lines of thought under current pursuit and some of the important physiologic questions which remain unanswered.

The term *ascites* was apparently introduced by Trevisa[95] in 1398 and was derived from the Greek word *askos*, meaning a bag. Historical evidence indicates that the Egyptians (circa 1500 B.C.) were aware of abnormal collections of abdominal fluid[12b] associated with disease of the liver. Erasistratos[12b] (250 B.C.) likewise noted the association of ascites with firmness of the liver. Abdominal paracentesis to rid the patient of offensive fluid was advocated by Celsus about 20 B.C.,[98] and Paul of Aegina (7th century A.D.) treated ascites by drainage through a copper tube forced through the abdominal wall. He sternly warned against the unduly rapid removal of the fluid.[58b] In the many intervening years the phenomenon of ascites has been observed countless times although knowledge of its genesis was so scant that little could be offered the unfortunate patient other than to draw off the fluid through quills or cannulae.

OCCURRENCE OF CLINICAL ASCITES

Extensive clinical observation has shown that ascites commonly occurs in diseases associated with hepatic or portal venous congestion. Chief among these diseases is Laennec's cirrhosis of the liver. The familiar pathologic pattern leading to eventual portal fibrosis and marked distortion of intrahepatic vascular channels need not be described here.[55b] However, it should be emphasized that the hepatic deformity impedes the circulation through the organ with resultant portal congestion and possible ascites. In fact, the occurrence of ascites with portal cirrhosis is one of the most frequent and characteristic signs of the disease.[72] (Fig. 1.) But it is of equal importance that ascites may also occur when blood flow through the liver is impaired by hepatic venous obstruction the cause of which is quite extraneous to the liver. It stands to reason that long-standing congestive heart failure[13] from advanced mitral stenosis and tricuspid insufficiency, or from other causes of myocardial strain, can invoke unremitting venous engorgement which may seriously interfere with hepatic and portal blood flow. In like manner, chronic constrictive pericarditis[83] and distortion of the mediastinum with vena caval compression may similarly impede hepatic flow.[13] From the standpoint of the pathologic anatomy of the chronically congested liver, it is only necessary to recall that cellular atrophy, necrosis and eventual fibrosis occur about the central veins of the liver lobules, and the walls of the central

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veins may become thickened. Fibrosis to a lesser extent may occur in the portal spaces. Cut sections of these livers appear finely granular, and externally they assume the characteristic "nutmeg" appearance. This form of hepatic fibrosis has been called *congestive* or *cardiac cirrhosis*. It is apparent that the livers afflicted with portal cirrhosis, or congestive cirrhosis, have in common the impedence of blood flow through the liver vasculature. In either case portal hypertension or ascites may develop if vascular obstruction within the organ is critically severe.

Since liver disease with ascites is attended by portal congestion, it might be expected that isolated portal obstruction could likewise provoke ascites. Indeed, clinical observation has frequently indicated that variable degrees of ascites may result from portal occlusion due to endophlebitis (thrombosis) or external compression. However, systematic studies of the ascites arising from portal vein occlusion are notably few.

Ascites has been encountered less frequently in a number of disorders which are not associated with hepatic congestion or fibrosis. Severe hypoproteinemia, as in the nephrotic syndrome or starvation state, may provoke ascites. Less often carcinomatosis, tuberculous peritonitis, Meig's syndrome and myxedema are accompanied by this condition.

This discussion will be limited to a consideration of the ascites occurring with hepatic fibrosis and congestion. In order to present all of the important evidence, a consideration of experimentally produced ascites will be helpful.

EXPERIMENTAL ASCITES

As with many forms of clinical and physiologic investigation of human disease, supplementary knowledge of value may be gained from animal experimentation performed under careful control. Reported studies of experimental ascites, when regarded *in toto*, comprise one of the interesting chapters of general pathologic physiology.

Earlier investigators produced artificial ascites by introducing various solutions directly into the peritoneal cavity of the experimental animal. They were able to study the physiologic results of increased intra-abdominal pressure and to observe certain factors controlling body fluids.[9,17,19,70,89,94] In general, these studies indicated that the introduction of either hypo- or hypertonic solutions into the peritoneal space evoked fluid adjustments rendering the instilled fluid osmotically equal to the plasma before resorption occurred. With few exceptions, these data were obtained from normal animals with no lesions to sustain the "ascites" and the data were therefore limited to fluid balance studies in a serous space.

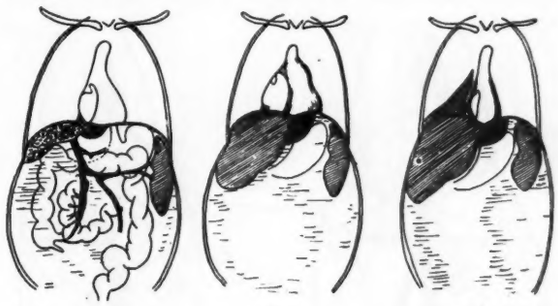
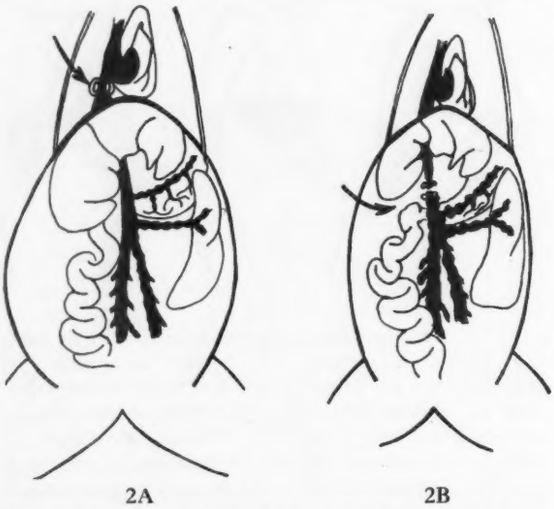


FIG. 1. Diagrams illustrating common conditions causing ascites. A, cirrhosis; B, hepatic congestion; C, pericarditis, mediastinal disease and hepatic congestion. It is notable that these conditions produce disturbances of intrahepatic circulation, whether secondary to hepatic fibrosis or chronic congestion of the liver (ultimately producing fibrosis) as seen in heart failure or distortion of the mediastinum with impairment of hepatic venous drainage.

"Cirrhosis" from Embolic or Toxic Injury of the Liver. The clinical occurrence of ascites with intrinsic liver disease suggests the value of studying experimental "cirrhosis." Repeated administration of small amounts of carbon tetrachloride to dogs or smaller laboratory animals may produce hepatic cellular deterioration with temporary elevation of portal venous pressure.[8,18] Hepatic fibrosis occurring from high fat, low protein diets in small animals is likewise well known.[36,68] In addition, fibrotic damage of the liver may be provoked by tannic acid administration,[48] portal embolism of the liver,[75] experimental biliary cirrhosis[8] or by the administration of radioactive gold.[41] These methods have been primarily employed to produce portal hypertension, with little attention given to the possibility of ascites formation occurring in the wake of these lesions.

Methods of Provoking Hepatic Engorgement. Experimental hepatic congestion has been the most successful means of inducing sustained ascites. Certainly it is an old method. Richard Lower in 1669[53] was the first observer to show that ascites developed in dogs following partial ligation of the inferior vena cava above the diaphragm. Subsequently, little was accomplished in this direction until Charles Bolton[9] studied the

effects of pericardial constriction and thoracic inferior vena caval obstruction in the genesis of peripheral tissue edema and, quite incidentally, of ascitic fluid formation. Other workers[1,11,23,32,55] have since used similar methods to induce peripheral and hepatic venous congestion in order to study the causes of ascites. The results were inconsistent in many of these studies. On the other hand, the experiments of McKee and his associates.[58a,76] seem particularly noteworthy. They were able to show that a reduction of the lumen of the inferior vena cava in dogs by about one-half, using pliable aluminum bands placed immediately above the hepatic veins, nearly always provoked massive ascites. This general procedure has been extensively employed by others;[5,40,93,97] cellophane bands and polyethylene wrappings have also been used to narrow the thoracic inferior vena cava. However, on the basis of a number of reports the aluminum band technic used by McKee et al. appears to be the simplest and most reliable method. Dogs which have been subjected to thoracic inferior vena caval stricture by bands generally develop ascites one or two weeks following the operation.[5,51,58a] (Fig. 2.) Moreover, the ascites will frequently persist for some four to six months and later disappear spontaneously. Occasionally the fluid has been observed to persist for one to two years without further surgical intervention.[51,58a,93] Transient portal hypertension may follow vena caval constriction; the duration of the hypertension appears to hinge on the rapidity with which collateral portasystemic communications develop.[93]



2A 2B

FIG. 2. Diagrammatic sketches showing two methods of provoking ascites in dogs. A, constricting band placed on the thoracic inferior vena cava above the hepatic veins; the liver is enlarged with distention of the portal venous system. Ascites produced in this way is intensified by the administration of salt; it may persist for long periods. B, this shows that the portal vein has been ligated and divided near the liver (in practice, portal vein is constricted gradually by surgical means over a period of weeks before final division of the trunk, to allow development of collateral venous circulation). Portal venous ligation and division usually does not produce ascites; when combined with plasmapheresis a more "serous" type of ascites will occur. Liver is not congested; portal veins distended below ligature.

When ascites has thus been established in the experimental animal, there is rapid passage of plasma protein into the ascitic fluid. This event has been designated "internal plasmapheresis."[58a] The term seems applicable because the loss of protein into the ascitic fluid occurs without detectable alteration in blood volume. In addition, the chronic "ascitic" dog preparation (thoracic inferior caval constriction) shows little evidence of impaired hepatic function as judged by bromsulphalein clearance and the regeneration of plasma proteins.[5,58a] In fact, these "ascitic" animals usually display normal physiologic functions except for the tendency to form ascites.

From this brief description of methods utilized in the production of experimental ascites it is evident that pathogenic factors may prevail which are similar to those occurring in clinical ascites secondary to portal or congestive cirrhosis. Consequently, it would appear logical to analyze the various factors which may influence ascitic fluid formation using both clinical and experimental information.

FACTORS INFLUENCING ASCITES FORMATION

General Principles of Fluid Exchange. Many years ago Starling[86a] described the basic principles controlling the exchange of fluid between the blood stream and tissue spaces. In spite of claims to the contrary, Starling's principles of fluid balance, when applied to the actual passage of fluid to and from the capillary seem as valid today as at the turn of the century.

In brief, Starling's principles state that the capillary hydrostatic pressure and colloidal osmotic force of the tissue fluid favor movement of fluid into the perivascular spaces, whereas the colloidal osmotic force of the blood together with tissue hydrostatic pressure act to retain fluid within the vasculature. The importance of capillary permeability and the lymphatic drainage in the regulation of these fluid transfers was

also taken into consideration. Other studies[50] have further refined this knowledge in terms of precise quantitation of the forces concerned in this delicate balance. A scheme of this familiar mechanism is shown in Figure 3. Although the blood crystalloids exert the main osmotic force, their free passage into the tissues essentially negates their role in fluid transfers. However, the capillary wall is relatively impermeable to plasma protein so that the osmotic force of the plasma proteins assumes great importance in opposing the filtration of fluid at the arterial end of the capillary and in recalling fluid from the tissue spaces at the venous end. Normally very little protein escapes from the capillary wall, and the small quantity that does is swept from the tissue spaces by the lymphatic channels which are permeable to protein molecules. It follows that elevation of capillary filtration pressure from venous congestion may provoke tissue edema by overwhelming both the osmotic system of fluid removal as well as the lymphatics. Conversely, the loss of plasma proteins from the vascular stream will nullify the optimal removal of fluid from the extravascular spaces so that tissue edema is again the result.

Influence of Portal Hypertension. It has been widely held that ascitic fluid, like edema fluid elsewhere, is the result of increased fluid filtration into the peritoneal space under the impact of portal hypertension generated by hepatic disease or generalized severe venous congestion. If one considers this problem well, proof for the contention must lie in the demonstration of significant portal hypertension in cases of ascites.

The portal system is unique in that it originates in the mesenteric capillaries and terminates in the hepatic sinusoids, and is therefore inaccessible for routine pressure measurement. Direct recordings of portal tensions in man have been secured during laparotomies[7,75,88] or through heparinized catheters retained in the portal vein following surgery.[81] One group of workers[26] reported portal pressure measurements by the passage of a catheter through an Eck fistula. Others[6] have attempted to record the pressure by direct puncture of the portal vein from the exterior of the body, and by cannulation[20] of collateral veins in the abdominal wall. Bean and his coworkers[3] reported that the distention of a balloon within the rectal lumen will blanch the mucosa at a tension essentially equal to that of the portal capillaries. Recent experimental studies have also indicated that "wedge pressures" obtained by catheterization of the hepatic veins of dogs may be nearly equal to portal venous pressure.[34a,60] This method may prove practicable for clinical use. Fortunately, direct portal pressure measurements have been secured during laparotomy in a number of patients with presumably normal visceral circulation.[4a,39,65]

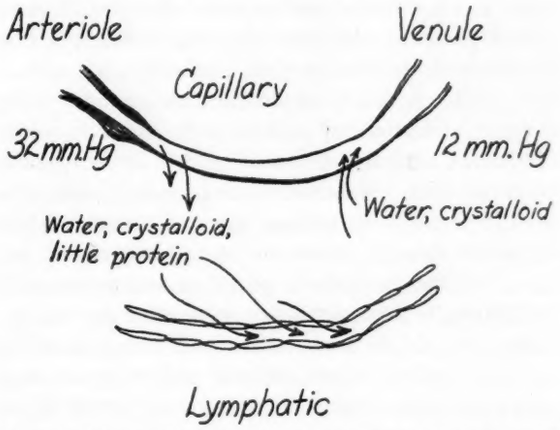


FIG. 3. Schematized drawing to illustrate basic principles of fluid balance, as affected by capillary filtration and osmotic forces. Ordinarily, the additional factors of tissue fluid pressure and osmotic force of tissue fluid proteins are negligible and are omitted from the drawing. See text.

These recordings show that *normal* portal pressures in man, under conditions of anesthesia and laparotomy, vary from 13.0 to 23.5 cm. of water. This range of normal pressure is essentially corrobrated by various indirect methods of measurement. Most patients with Laennec's cirrhosis exhibit moderate elevation of portal tension, usually 22.0 to 35.0 cm. of water.[39,65] Obviously, there is some overlapping of the lower portal pressures observed in cirrhotics and "normal" pressures in individuals having abdominal operations. Perhaps these so-called "normal" pressures are the result of the development of an adequate portavenous collateral circulation. Occasionally the portal pressure in cirrhosis may be more elevated (50.0 cm. of water or more).[88] Nevertheless, the usual reported elevations of portal pressure in hepatic cirrhosis are such that there exist only nominal increases in terms of presumed splanchnic capillary pressure, so that the actual filtration force applied to the capillary membrane may not be extreme.

Numerous observations, experimental and clinical, have shown that the presence of detectable collateral portasystemic venous communications (e.g., esophageal varix, dilatation of

abdominal veins and hemorrhoids) is not necessarily pathognomonic of existing portal hypertension.[44a] It seems more probable that these collateral vessels limit the severity of portal hypertension and prevent excessive pooling of blood in the splanchnic bed. Of great importance are the repeated observations that there may be little or no correlation between the elevation of portal pressure and the occurrence of ascites. For example, it is known that patients with occlusion of the portal vein without ascites may have greater elevation of portal pressure than some cirrhotics with marked ascites.[75,88] In fact, some patients with cirrhosis exhibit ascites when the portal tension is within normal limits, while other cirrhotics show no abdominal fluid yet have marked increase in portal venous pressure.[88]

Although these data argue against the importance of portal hypertension in the genesis of ascites, certain experimental observations suggest that some forms of ascites may result from increased portal tension. If partial or complete occlusion of the portal vein is carried out in dogs (with or without constriction of the abdominal vena cava above the renal veins), ascites will rarely occur even when large quantities of sodium are administered.[5,93] However, if severe hypoproteinemia develops, or if plasmapheresis is combined with the procedure, ascites formation is generally induced.[93,97] But this ascites is transient and the ascitic fluid contains very little protein. This is in marked contrast to the voluminous ascites following vena caval constriction, this fluid being rich in protein, persistent, and often associated with normal portal pressure.[93] The ascites in the former case (i.e., portal occlusion with hypoproteinemia) seems dependent upon augmented portal capillary pressure together with failure of osmotic fluid recall incident to hypoproteinemia. It seems possible that a similar mechanism may be responsible for the ascites noted in certain clinical cases of portal venous occlusion although the frequent association of chronic liver disease in these cases clouds the issue.

In summary, the present evidence suggests that portal hypertension is not the dominant factor in the pathogenesis of the voluminous ascites encountered in Laennec's cirrhosis or in the experimentally produced ascites of hepatic congestion. Portal hypertension may be contributory to ascites in instances in which critical elevation of pressure is present, particularly when the plasma proteins are so reduced as to nullify the normal osmotic force. In subjects with portal cirrhosis or portal obstruction from other cause, the development of collateral portasystemic communications may account for the maintenance of portal tension within normal or slightly elevated range.

Role of Plasma Proteins in Ascites Formation: Osmotic Pressure. It is known that the colloidal osmotic pressure of the blood is exerted primarily by the plasma proteins and particularly by the albumin fraction, due to its smaller molecular size and greater concentration.[38] Hence, the relationship of plasma protein levels to ascites formation requires careful examination.

Ascitic fluid resulting from portal cirrhosis or hepatic congestion is essentially similar to plasma except for a lower protein content. However, the protein itself is electrophoretically similar to that of the plasma.[54,58a] Although the protein levels of these ascitic fluids may vary widely, i.e., from 0.3 to 4.7 gm. per cent,[14,28,54] the average protein range is well above that seen in the subcutaneous edema of congestive failure (average, 0.24 gm. per cent).[86b] In some clinical and experimental instances, the protein content of ascitic fluid, as well as its volume, varies in proportion to the plasma protein levels.[56] For example, Ricketts[74] noted in a number of patients with cirrhosis that those with ascites showed plasma albumin levels below 3.0 gm. per cent; in cirrhotic patients without ascites the plasma albumin fractions were more nearly normal. But other workers[66,71] have reported that in similar patients no constant correlation exists between the blood colloidal osmotic pressure and the rate and volume of peritoneal fluid accumulation. In this connection it has also been demonstrated that, when cirrhotic patients with ascites are given concentrated salt-poor albumin, a transient elevation of plasma albumin level occurs but generally without any measurable effect on the rate of ascites formation.[35,64,90] On theoretic grounds it might be supposed that because ascites is possessed of generous protein content, this fluid might exert appreciable colloidal osmotic force to retain fluid in the peritoneal cavity. However, following the intraperitoneal injection of salt-poor albumin[49] significant elevation of serum albumin may take place with no increase in ascitic fluid volume; indeed, there may be an actual decrease in the quantity of this fluid.

From these observations it would appear that

the role of the oncotic pressure exerted by the plasma or the ascites in governing fluid accumulation is not entirely clear. Certain animal observations have added useful information. In carefully controlled experiments with "ascitic" dogs, McKee et al.[58a] demonstrated that when the animals were given low-salt, low-protein diets, ascitic fluid accumulation was rapid; furthermore, when the plasma protein level was raised by increased dietary protein intake with continued salt restriction, fluid formation was significantly retarded. But in the same animals when the plasma protein levels were raised (in some instances from 4.0 to 7.0 gm. per cent) by intravenous infusions of dog plasma, the ascites continued to form rapidly and the fluid contained large amounts of protein. These authors were also able to correct hypoproteinemia by infusions of amino acids in saline or distilled water, but only when the protein was given in distilled water was the ascites formation effectively halted. Since the fluid accumulation was *not* regularly prevented by increasing the plasma proteins, it was reasoned that the sodium content of the infused plasma or of the saline-amino acid mixture may have enhanced the output of fluid. As a matter of fact, in other studies these workers demonstrated that sodium retention may be the *sine qua non* in this form of ascites, the plasma protein being of secondary importance.

To summarize, it seems evident that the plasma and ascitic fluid protein levels are not the dominant factors in the regulation of fluid passing to the peritoneal space. However, the colloidal osmotic pressure of the plasma appears to exert a secondary influence on ascites formation.

Significance of Intra-abdominal Tension. The accumulation of ascitic fluid may render the abdomen extremely taut with intra-abdominal tensions varying from 15 to 60 cm. of water. If this pressure represents a tissue hydrostatic force, it may be questioned whether it opposes the entrance of fluid into the peritoneum. Studies by Mankin and Lowell[56] and James[46] suggest that the magnitude of this force is actually governed by the rate of ascites formation, and that it exerts only minor influence on the mechanism of ascites production.

It is true that a large collection of fluid in the abdomen leads to elevation of inferior vena caval pressure and may contribute to the edema of the lower extremities often observed in cirrhotic patients with ascites.[20] It has been stated that there is likewise an associated elevation of right atrial pressure in such instances[23,77] although this finding has been disputed. Furthermore, the possibility of general venous hypertension influencing salt and water retention is unsettled. Nevertheless, it is striking that following paracentesis, ascites may form at increased rate in spite of a normal venous pressure.

The remarkable capacity of the peritoneal cavity to accommodate as much as 15 L. of water is always a source of wonder. It is known that sudden distention of the peritoneal space with fluid may precipitate shock and death.[89] Slowly increasing distention may be tolerated for long periods, with consequent stretching of the abdominal wall to a striking extent.[16]

In summary, these data suggest that elevation of intra-abdominal tension may influence ascites formation, but to a minor extent only.

Effects of Sodium and Water Retention in Ascites. The evidence to this point indicates a conspicuous lack of correlation between ascites formation and alterations in portal venous pressure, plasma and ascitic fluid protein levels and tissue hydrostatic force, or any combination of these factors. With continued clinical and experimental study of the problem it has become increasingly apparent that the influence of sodium and water retention (as with edema in general) is of particular importance in ascites, once the optimum conditions of hepatic fibrosis or congestion are established.

Herringham and Hadfield[42] were among the first to observe the scanty excretion of salt in the urine of patients with ascites. Indeed, it is unusual when this is not the case.[49] Farnsworth and Krakusin[30] demonstrated a marked inability of cirrhotic subjects with ascites to excrete sodium. In the presence of ascites they noted that sodium retention was not associated with increased plasma sodium levels. These investigators also showed that a similar phenomenon of "sodium saving" occurred in subjects with chronic congestive heart failure. They suggested that whatever the fundamental mechanism of salt retention may be, it is probably the same in either cirrhotic or cardiac patients. Eisenmenger and his co-workers[27] found that patients with cirrhosis (on a normal salt intake) may eliminate as little as 1 mEq. (23 mg.) of urinary sodium per day. Other patients subjected to salt restriction (15 to 17 mEq. per day), but with high protein diets, showed a gradual increase of urinary

sodium excretion and urine volume, together with a concomitant rise of plasma sodium and diminution of ascites over long periods. Surprisingly enough, a number of these patients were ultimately able to take usual daily quantities of salt without the reformation of ascites. The reasons for this return to apparent normal sodium metabolism are not clear, although it seems likely that marked improvement in liver function must have been concerned.

Similar balance studies in dogs with ascites exhibited remarkably parallel results. Animals with thoracic inferior caval stricture[23,76] or with ascites from experimental constrictive pericarditis[23] demonstrated consistent failure of optimum urinary sodium excretion. Moreover, McKee and his ascociates[58a] found that marked dietary sodium restriction in the "ascitic" dog strikingly reduced the rate of ascites formation even in the presence of severe hypoproteinemia. These observations seem adequate to illustrate that sodium wields an important influence in the pathogenesis of ascites.

It is not possible to discuss here the numerous studies concerning the hormonal control of sodium and water excretion. Inclusive reviews of this material include those of Eisenmenger and others.[27,82] However, a few facts may be repeated with profit.

At present, much of the experimental evidence indicates that renal sodium excretion is not necessarily controlled by glomerular filtration rate nor by renal blood flow either in the normal state or in clinical conditions associated with edema.[23,30,37] These facts are also evident in the experimental animal with pericardial constriction or vena caval obstruction.[23,84] Nor has definite correlation been found between salt excretion and a falling cardiac output.[12a,21] Rather, most evidence points to the renal tubular resorption of sodium as the main custodial influence upon this ion. Furthermore, this sodium-saving device apparently operates independently of posterior pituitary antidiuretic hormone.[67] It is of further interest that the kidneys themselves do not determine the extent of salt and water retention but are rather blindly subservient to demands for the retention of these substances whether the body will benefit or not. Therefore, conditions entailing shifts in fluid compartments, as seen in cirrhosis or general venous engorgement, may evoke stimuli to salt and water retention to which the kidneys are instantly tuned.[67] The exact origins of these stimuli are not known but certain possibilities warrant mention.

The evidence suggests that adrenal cortical hyperactivity may occur in conditions characterized by general edema and ascites.[10] These data are of importance in the light of inferential evidence that certain adrenal steroids may wield sharp influence on the renal excretion of sodium. As an example, in the "ascitic" dog cortisone and desoxycorticosterone acetate had little effect on ascitic fluid formation.[23,24] On the other hand, when these animals were subjected to bilateral adrenalectomy marked sodium excretion and diminution of ascites occurred in spite of a persistently elevated vena caval pressure. It was only after the ascites was gone that evidence of adrenal insufficiency became outspoken. The adrenalectomized animals could then be maintained in good health on the usual maintenance doses of DCA (0.5 mg. per day). Ascites did not recur on this schedule, nor when cortisone was given in large amounts.[24] Only when DCA was administered in large doses (10 to 25 mg. per day) did the degree of sodium retention and ascites formation equal that of the intact "ascitic" dog. Therefore, DCA-like hormones appear to be essential to this type of ascites formation.

Many patients with cirrhosis of the liver and ascites exhibit a reduction of sodium excretion in the saliva, sweat and feces[4b,27] as well as in the urine. Relative increases in potassium concentration in these non-urinary excreta are likewise encountered. Since similar alterations have been produced by the administration of large amounts of DCA, it is possible that increased activity of the adrenal mineralocorticoids may lead to the electrolyte shifts noted in cirrhotic patients.[23]

Another factor in the salt and water retention observed with ascites may reside in the posterior pituitary antidiuretic hormone.[71] Numerous studies have shown that this hormone probably acts directly upon the renal tubules, quite independently of sodium retention. The studies of Verney[92] have demonstrated a relationship between antidiuretic hormone and serum sodium concentration. He suggested that elevation of serum sodium induces increased elaboration of this hormone, while depression of sodium level inhibits the formation of the hormone. Ralli and her associates[71] were among the first observers to show that an increase in an antidiuretic factor often occurred in the urine of patients with cirrhosis and ascites. They

originally postulated that failure of the damaged liver to inactivate this substance was important in speeding the rate of ascites accumulation. However, others[79,91,93] have not been able to demonstrate the consistent presence of this substance in the urine of patients with ascites or in the "ascitic" dog. Nevertheless, definite alterations in water metabolism in cirrhotic subjects undoubtedly occur.[71] Delayed excretion of a test dose of water appears in the ascitic patients and to a lesser extent in those without ascites. These aberrations may be secondary to altered activity of the antidiuretic hormone. Suffice it to say, the role of the antidiuretic hormone in edematous states in general remains to be clarified.

It has likewise been postulated that, under conditions of critical parenchymal damage, the liver may fail to inactivate the hormones of the adrenal cortex and posterior hypophysis as well as certain sex hormones, which possess some salt-retaining properties.[82] The liver has been further implicated by evidence[78] that a biologically active material, presumably liberated by the liver (VDM), has certain antidiuretic properties. This material seems to be excreted in increased quantities in patients with ascites or cardiac failure. The role of VDM in the genesis of ascites is not clear.

One other mechanism should be mentioned relating to salt and water retention. Under some conditions, the critical elevation of venous pressure is said to induce sodium retention without the antecedent formation of edema.[34b] From the experimental evidence it seems unlikely that this factor is important in ascites formation. Constriction of the portal vein, with resultant hypertension of the splanchnic bed, has little effect on sodium excretion.[76] Furthermore, constriction of the abdominal vena cava below the diaphragm fails to produce permanent alterations in sodium excretion despite elevation of renal venous pressure.[44b,84] On the other hand, hepatic congestion is generally associated with persistent sodium retention and ascites formation even though renal hemodynamics return to normal.[84] In fact, hepatic congestion unaccompanied by vena caval hypertension is sufficient to provoke ascites and presumably sodium retention.[97] These apparent inconsistencies in the handling of sodium and water are confusing but there is strong evidence that the congested liver possesses a unique ability to alter salt and water movement[23,76] which is not seen in congestive states involving other areas of the body.

In summary, there appears to be little doubt of the importance of the sodium ion in the genesis of ascites incident to hepatic fibrosis of the portal or congestive type. Sodium excretion is governed primarily by the renal tubules. However, the mechanism by which salt and water retention occurs in ascites requires clarification, although important data have been obtained.

ORIGIN OF ASCITIC FLUID

One might assume that ascitic fluid merely transudes from the splanchnic capillaries by the simple process of filtration subsequent to portal hypertension and lowered plasma colloidal osmotic pressure. Examination of the experimental and clinical data has shown that the genesis of ascites is more complex. In the first place, in the few instances in which accurate measurements of portal venous pressure have been made at laparotomy, the degree of portal hypertension in Laennec's cirrhosis may be insufficient to account for a copious transudation from the splanchnic bed. Second, the quantity of protein in most ascitic fluids is appreciably greater than that found in the superficial edemas of congestion or hypoproteinemia. From these factual data there is little foundation for the argument that the usual type of ascitic fluid is a simple capillary filtrate. It is now necessary to make a systematic inquiry into other possible sources of this fluid.

The experiments of Schilling et al.[76] and of Milnes[59] on dogs and rabbits indicated that portal venous occlusion alone did not provoke ascites. However, when the thoracic inferior vena cava was then constricted, a voluminous ascites formed similar to that in the "ascitic" animal. This not only indicated the importance of hepatic congestion but clearly pointed to the liver as the possible source of ascitic fluid.

The results of these and other studies have suggested that ascites is derived directly from hepatic lymph. It is true that lymph collected from the channels close to the liver hilum contains more protein than lymph from other sources.[57] Drinker and his co-workers[31] found in the normal dog that, with a serum protein content of about 6.3 gm. per cent, the protein content of the liver lymph averaged 5.3 gm. per cent in comparison to a level of 1.91 in the lymph from the legs and 3.9 gm. per cent in the lymph from the small intestine. It would seem, therefore, that the intrinsic capillaries of the liver are extremely permeable to protein, a fact which

was recognized by Starling many years ago.[86a] Furthermore, Nix and his associates[62] found in dogs and rats a marked augmentation of hepatic lymph flow when this organ was subjected to venous congestion or to carbon tetrachloride cirrhosis. The increment in lymph flow, always seen prior to ascites formation, was apparently not accompanied by increased lymph drainage from the other abdominal viscera.[8] These workers suggested that ascitic fluid extravasated from the extrahepatic liver lymphatics. From collected studies Gray[39] postulated that during hepatic congestion the production of lymph may be so brisk as to overwhelm the lymph channels, with extrusion of the substance into the peritoneal space. However, there is, to our knowledge, no experimental proof that extravasation of hepatic lymph occurs through the intact walls of the larger extrahepatic lymph vessels. But it does seem possible that when voluminous lymph production occurs, as from engorgement of any tissue, the limited carrying power of the lymphatic vasculature may compel the extrusion of the fluid directly from tissue spaces. Therefore, the question is quite naturally raised as to whether egress of fluid from the liver itself is the immediate source of the bulk of ascitic fluid.

Other observations lend very real credence to such a suggestion. An example is provided by studies of pulmonary engorgement. When the outflow of pulmonary venous blood is progressively impeded by experimental myocardial insufficiency,[63] there is an immediate augmentation of lymph flow from the right thoracic duct. If pulmonary congestion becomes severe, alveolar transudation appears even though lymph flow has increased several fold. Concurrently with the appearance of pulmonary edema, fluid forms on the pleural surface of the lungs and escapes in numerous large drops. A similar phenomenon may be observed in the myocardium. The imposition of sudden, marked strain by the creation of acute aortic insufficiency or stenosis may be accompanied by signs of myocardial dilatation and acute failure; in addition, drops of fluid will appear on the epicardial surface so that the membrane is constantly moist.[80]

That the liver may be a major source of fluid in the generation of ascites is supported by direct evidence from other studies. Many years ago, when Bolton constricted the inferior vena cava in the thorax to produce ascites in experimental animals, he noted the presence of endothelial proliferations of the liver capsule. He suggested that these proliferative areas could represent the site of fluid formation contributing to ascites, together with fluid from mesenteric capillary filtration. Since then a number of investigators have taken note of subcapsular lymphatic dilatations associated with congestion of the liver[58a,93] from which fluid might be expected to exude during hepatic engorgement. Freeman[33] reported the interesting observation that when the dog liver was surgically transferred to a supradiaphragmatic position and then congested by thoracic caval constriction, the ascites that formed collected in the thoracic cavity alone. Unfortunately, no other data were given regarding these unpublished observations. Hyatt, Lawrence and Smith,[45] studying the "ascitic" dog, have extended these observations. After ascites had developed in these animals they were subjected to laparotomies with wide exposure of the abdominal viscera. The livers were quite evidently congested. Most striking was the undoubted constant formation of drops of fluid on the surface of the livers. By placing nonirritating cellophane bags beneath the left hepatic lobes, uncontaminated samples of "liver fluid" were collected for analysis. It was interesting that during long periods of exposure the hepatic surfaces remained moist whereas the exposed surfaces of the other abdominal viscera were comparatively dry. In Table 1 are listed the protein determinations of some representative fluid samples collected during these experiments. It was also possible to collect samples of fluid from the liver lymphatics. Since it has been shown that lymph is essentially equivalent to tissue fluid in composition, the general similarity between the liver lymph and the "liver fluid" was not unexpected.

TABLE 1
PROTEIN CONTENT, EXPRESSED IN GM. %, OF REPRESENTATIVE FLUID SAMPLES OBTAINED FROM TWO "ASCITIC" DOGS (CONSTRICTED THORACIC INFERIOR VENA CAVAE)

Dog	Plasma	Ascitic Fluid	"Liver Fluid"	Liver Lymph
No. 5.....	3.6	2.0	3.4	3.4
No. 6.....	4.9	3.3	4.5	4.5

The observations of an augmented liver lymph flow, the high protein content of liver lymph and

ascitic fluid, and the exuding of a proteinous fluid from the liver surface strongly suggest that in the experimental ascites of hepatic congestion (and possibly hepatic fibrosis), some of the water and much of the protein of ascitic fluid are derived from the liver capsule.[45,93] Additional water may enter from other serous surfaces. Although confirmatory clinical studies are lacking, this concept of ascitic fluid formation may prove applicable to the ascites associated with portal or congestive cirrhosis in man.

The inability to correlate plasma colloidal osmotic pressure and capillary hydrostatic forces (represented by portal venous pressure) with ascites formation has cast doubt on the validity of Starling's principles here. However, if this concept of ascites origin is valid, the capillary exchanges incident to the fluid formation occur primarily within the liver. Therefore, the usual measurement of portal venous pressure is probably not representative of the intrahepatic capillary hydrostatic force. Furthermore, the extreme permeability of the hepatic capillaries suggests that copious fluid filtration may occur with comparatively minor increments of intracapillary tension. These considerations warrant that a complete evaluation of intrahepatic fluid exchange be made in terms of these types of ascites formation before Starling's principles are cast aside.

Dynamics of Ascites Formation. The question now arises as to the steps leading to the accumulation of peritoneal fluid. Severe, long-standing hepatic stasis from congestive or portal cirrhosis leads to distortion of the intrahepatic circulation and sets the stage for escape of fluid into the peritoneal cavity. At some point in the process circulatory homeostasis is altered to the extent that salt and water retention occur. While the nature of the receptors controlling these alterations is unknown, the action of both volume and osmoreceptors has been postulated. Moreover, it is not clear whether these reactions curtailing water and sodium excretion are brought into play before the ascites accumulates, or whether ascites, with its possible dehydrating effect on the circulation, occurs in advance. However, there is some indication that ascites formation begins prior to sodium retention in the "ascitic" dog.[23]

Once this stage is reached a vicious cycle apparently operates. As sodium and water are lost to the abdominal cavity, further retention of these substances occurs and there is further filtration of fluid into the ascitic pool possibly from the liver capsule. As additional insult, the loss of large quantities of plasma protein into the ascites lowers the effective osmotic pressure of the blood, favoring even greater fluid accumulation. The exact role of hepatic congestion in determining this behavior of fluid dynamics is in doubt. Perhaps as Schilling et al.[76] suggested, the mechanism is related to the unique anatomy of the liver lobule which may render the organ extremely susceptible to minor changes in venous pressure and stasis. Be that as it may, these data permit the guarded speculation that liver congestion *per se* may be directly concerned in initiating the fluid and electrolyte disturbances observed in congestive heart failure. This possibility should receive careful investigation in the future.

Although accumulated peritoneal fluid appears as a static pool, there is information that ascitic fluid is actually in dynamic equilibrium with the blood. By means of radioactive-tagged plasma protein, McKee et al.[58a] demonstrated a complete turnover of ascitic fluid albumin in approximately two days; they found that globulin exchange was somewhat slower. Furthermore, Prentice and associates,[69] using tritium-labelled water in a study of ascitic patients, estimated that from 40 to 80 per cent of the total ascitic volume enters and leaves the peritoneal cavity each hour. For example, a patient with 6 L. of ascitic fluid may circulate from 58 to 115 L. of water through the abdomen in each twenty-four-hour period.

The problem of the disappearance of ascitic fluid is no less interesting. In the "ascitic" dog preparation release of vena caval constriction will lead to prompt disappearance of ascites, provided no permanent narrowing of the vessel has occurred. Abnormalities in sodium metabolism and evidences of hyperfunction of the adrenal cortex also disappear.[23] Occasionally, spontaneous disappearance of ascites may occur in animals with persistent hepatic congestion. The explanation of this phenomenon is not clear, although it has been suggested that adrenal cortical exhaustion may be concerned. On the other hand, the gradual development of an adequate collateral venous circulation may hasten the relief of hepatic-portal venous stasis in the experimental animal and possibly in man. Finally, the restriction of sodium may be impressively effective in abolishing ascites and is therefore an important therapeutic suggestion.

BRIEF CONSIDERATIONS OF THERAPY

It is evident from the preceding discussion that there are a number of physiologic occurrences which suggest a rational approach to the treatment of ascites. These therapeutic considerations will be described briefly, and only in sufficient detail to signify their value. Detailed accounts of treatment may be obtained from papers in the accompanying bibliography.

When cases of Laennec's cirrhosis or congestive cirrhosis become advanced, with irreversible pathologic changes, the resultant ascites is frequently difficult to control. Nevertheless, some of the more fortunate patients with portal cirrhosis show "spontaneous" restoration of hepatic compensation from rest, nutritional support and adequate attention to fluid balance. In conditions of chronic hepatic engorgement from less tractable congestive failure, the ascites may be relieved by eventual improvement in myocardial function. There is now justification for the hope that some measure of prevention of congestive cirrhosis may accrue as surgical procedures for the correction of mitral stenosis and other chronic valvular defects become more widely used. There are also numerous recorded instances in which surgical relief of chronic constrictive pericarditis was accompanied by loss of ascites.

Once irreparable hepatic changes have occurred and the formation of ascites established, treatment may be effective when directed toward the physiologic disorders previously indicated by clinical and experimental observations. Removal of ascites by paracentesis in mandatory when the accumulation is excessive. However, it should be recalled that ascitic fluid contains substantial quantities of protein so that frequent drainage represents severe protein waste. For instance, the removal of 4 L. of ascites with a protein content of 2 gm. per cent represents a total loss of 80 gm. of protein. In addition to protein loss, paracentesis may remove considerable amounts of sodium. Indeed, the sodium loss may be so marked as to contribute to the low serum sodium levels frequently encountered in these patients, and may even induce symptoms of hyponatremia.[61]

It will be recalled that one of the prominent influences in the formation of ascites from hepatic disorders is the lagging elimination of salt and water. There is impressive clinical evidence that careful restriction of sodium chloride in the diet may significantly retard the reaccumulation of ascites or prevent its reappearance for long periods.[27,30,47] Layne and Schemm[52] studied sixteen patients with hepatic cirrhosis and ascites who were given low salt diets and comparatively liberal fluid intake (though supplemented with mercurial diuretics and ammonium chloride) and reported the clearing of ascites in all but one. Kunkel et al.[49] limited salt intake to less than 1 gm. per day in thirteen patients with ascites. Fluid formation stopped in twelve of the thirteen subjects. These observers also reported that their patients were able to consume as much as 150 gm. of protein per day, and that the improvement may have been due to part to cessation of protein waste from repeated paracenteses. Furthermore, in most of their patients ascites tended to reform when sodium intake was increased. The evidence from these and other reports indicates that the adequate treatment of ascites should include a reduction in daily salt intake to the lowest practicable levels. In addition, sodium elimination may be hastened by the judicious use of mercurial diuretics, with ammonium chloride when necessary. Sodium ion exchange resins are useful adjuncts in reducing sodium intake in patients with resistant ascites. It also stands to reason that an adequate protein intake should be sought in these patients, not only in an attempt to slow ascites accumulation but also to improve general nutrition. In this regard, Kark and his co-workers[47] have postulated that severe protein depletion may directly influence capillary permeability favoring augmented ascites formation.

The various surgical procedures devised to relieve the complications of portal hypertension need not be redescribed. Perhaps the most famous of these are the Eck fistulas and other variants of portacaval shunts. The procedures have occasionally been used with success in eliminating stubborn ascites. In recent years surgical operations aimed at changing the dynamics of the abnormal intrahepatic circulation have been under trial. A number of observers[5,73] have reasoned that since marked distortion of the intrahepatic vascular tree results from advancing portal cirrhosis, the elimination of hepatic arterial flow might lessen the severe element of stasis produced in the restricted portal bed. This reasoning was supported by the experiments of Dock.[25] He showed that when excised cirrhotic livers were perfused with kerosene, perfusion rates through the portal vein were enhanced by retarding, or stopping,

the hepatic arterial stream. This work implied that intrahepatic arteriovenous shunts were instrumental in raising portal venous pressure during life. The work further suggested that hepatic arterial ligation might be clinically beneficial in cases of portal hypertension due to fibrosis of the liver. In terms of practical surgery, relief of portal hypertension and even of ascites formation has been reported by use of this operation. However, Taylor and Rosenbaum[87] have expressed opposition to the procedure on the basis of their experimental and clinical observations that obliteration of hepatic arterial flow did not enhance the flow of blood through the portal vein. They believe that there may be more objections than merits to the operation. Further evaluation of this surgical approach is obviously required.

From the résumé, it is apparent that no single therapeutic procedure is uniformly successful in combatting ascites. Since ascites usually signifies serious disease, any rational therapy, even of heroic kind, seems quite justified.

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Clinico-pathologic Conference

Metastatic Carcinoma of Undetermined Primary Site

STENOGRAPHIC reports, edited by Robert J. Glaser, M.D. and David E. Smith, M.D. of weekly clinicopathologic conferences held in the Barnes Hospital, are published in each issue of the Journal. These conferences are participated in jointly by members of the Departments of Internal Medicine and Pathology of the Washington University School of Medicine and by Junior and Senior medical students.

THE patient, J. R. (No. 220439), was a Negro musician, seventy-three years of age, who entered the Barnes Hospital for the first time on March 12, 1953, complaining of pain in the right hip. The family history was non-contributory. The past history indicated that the patient had been in good health for most of his life. In his youth he had had gonorrhea several times; for many years he had had a chronic cough which had become more severe in the two years prior to admission. The cough was productive of white mucoid sputum which on occasion was blood-streaked. Eleven years before entry the patient injured his right ankle and soon thereafter the skin of that leg became sore, swollen and thickened. Two years before admission a papular, itching, desquamating eruption developed over the right leg; it subsequently spread to the thigh, the inguinal region and to the back. After the rash disappeared the skin in the involved area remained thickened and more intensely pigmented than the other skin.

Twelve years before entry the patient noted diminution in the size of his urinary stream. With this symptom were associated urgency, hesitancy and nocturia. The patient was forced to undergo urethral dilatation on a number of occasions and learned to catheterize himself in order to relieve bladder distention. His urinary symptoms gradually increased in severity. Six months before admission urination was followed by the passage of bright red blood. There was no concomitant pain and hematuria did not recur. Three months before entry the patient noted the onset of constipation and defecation became painful. The stools were firm and occasionally covered with bright red blood, but there had been no diarrhea or change in the caliber of the stools. The patient also noted constant, dull, aching low back pain which radiated down the posterior aspect of the right leg to the ankle. The pain was described as being "like a toothache." One month before admission the patient's right leg became numb and weak, and he stated that he felt as if he were "walking on a sponge." At the same time he noted large, non-tender nodules in the right inguinal region which slowly increased in size. Because of his symptoms the patient sought medical care. In the past seven years he had lost sixty pounds, one-third to one-half of it in the past two years.

Physical examination revealed the temperature to be 37°C., pulse 80, respirations 16 and blood pressure 140/90. The patient was a well developed Negro male in moderate distress, complaining of back pain. He appeared chronically ill and there was evidence of weight loss. Over the skin of the back were three firm subcutaneous nodules, 1 to 2 cm. in size, which were lobulated, sharply defined, freely movable and non-tender. The skin over the lower back, buttocks and thighs was thickened and desquamated, and pigmentation was increased. Examination of the eyes revealed that the pupils reacted well to light and accommodation. The fundi were normal except for minimal narrowing of the retinal arterioles. The upper respiratory tract appeared normal. The neck was supple. The trachea was in the midline. There was an increase in the anteroposterior diameter of the chest which was hyperresonant to percussion. A few coarse rhonchi were heard at both lung bases posteriorly. The heart was of normal size and the rhythm was regular. A soft, blowing, high-pitched systolic murmur was heard at the

apical area. Abdominal examination was negative. The testes were atrophic but the genitalia appeared otherwise normal. The prostate was two and one-half times enlarged and nodular. A firm, fixed, 0.5 cm. nodule was felt in the right lobe. There was atrophy and weakness of the hamstring muscles of the right leg and marked tenderness on pressure over the sacrum and over the right hip joint. Hyperesthesia was elicited over the lateral aspect of the right leg and straight leg raising on the right caused severe pain. The reflexes in the lower extremities were normal and there were no pathologic toe signs.

The laboratory data were as follows: blood count: red cells, 4,540,000; hemoglobin, 14 gm. per cent; white cells, 8,700; differential count: 2 per cent eosinophils; 35 per cent segmented forms; 61 per cent lymphocytes; 2 per cent monocytes. Urinalysis: specific gravity, 1.017; albumin, negative; sugar, negative; centrifuged sediment, occasional hyaline cast, five to eight white blood cells and occasional epithelial cell per high-power field. Urine smear: many gram-positive cocci; urine culture, coliform organisms. Stool: negative for occult blood. Blood cardiolipin test: negative. Blood chemistry: non-protein nitrogen, 22 mg. per cent; fasting blood sugar, 102 mg. per cent; total protein, 6.9 gm. per cent; albumin, 3.6 gm. per cent; globulin, 3.3 gm. per cent; alkaline phosphatase, 3 Bodansky units; acid phosphatase, 1.7 King-Armstrong units; sputum, numerous examinations negative for acid-fast bacilli. Roentgenogram of the chest: elongation of the thoracic aorta, old tuberculosis in the left apex and hypertrophic osteoarthritis of the thoracic spine. Roentgenogram of the spine: narrowing of the intervertebral spaces between third and fourth and fourth and fifth lumbar vertebrae and between the fifth lumbar and first sacral vertebrae. Electrocardiogram: abnormal form of ventricular complex. PPD skin tests: negative in first, intermediate and second strengths.

When the patient was admitted to the hospital, respiratory isolation was instituted; but these precautions were discontinued after multiple sputum examinations were negative for acid-fast organisms. The patient was given demerol® in an attempt to control his leg pain, but this drug produced hallucinations and had to be discontinued. A urologic consultant indicated that the nodule described on admission was an enlarged seminal vesicle; he made additional diagnoses of prostatitis, urethritis and urethral stricture from multiple gonorrheal infections. Intravenous pyelograms were performed and revealed a bifid left renal pelvis. A neurosurgical consultant suggested a diagnosis of tabes dorsalis.

On the fifth hospital day lumbar puncture was performed. The initial pressure was 230 mm. of water and the final pressure, after removal of 10 cc., was 100 mm. of water. The fluid was clear and contained only nine mononuclear cells per cu. mm. The protein was 80 mg. per cent, the chloride 120 mEq./L., and the Wassermann test negative. On the eighth hospital day lumbar puncture was repeated with cuff manometrics. Responses to the various pressures were normal. At this time the spinal fluid contained twelve lymphocytes, 117 mg. per cent protein and 120 mEq./L. chloride.

On the thirteenth hospital day myelography was performed by the Neurosurgical Service. Spinal fluid obtained on this occasion contained six mononuclear cells and 165 mg. per cent protein. The sugar was 14 mg. per cent, the colloidal gold curve first zone in type. The myelograms were unsatisfactory and were repeated several days later. The second attempt was successful; the films were interpreted as demonstrating herniation of the intervertebral discs between the fourth and fifth lumbar vertebrae and fifth lumbar and first sacral vertebrae. Osteoporosis in the region of the right sacral iliac joint and of the sacrum were noted and thought to be compatible with metastatic carcinoma, probably arising from the prostate. A repeat acid phosphatase determination was reported as 3.0 King-Armstrong units.

On the twenty-fifth hospital day the patient's temperature suddenly rose to 39.2°C. and he had a shaking chill. Examination of the chest revealed dullness to percussion over the left lower lobe; coarse rhonchi and moist rales were heard over the left chest up to the level of the scapula. A few rales were heard at the right lung base posteriorly. Sputum culture revealed type 6 pneumococci but blood cultures were negative. The white blood cell count was 18,700. A roentgenogram of the chest revealed linear streaking, extending from the left hilum to the left upper lobe. This finding had been present on earlier films but had not been commented upon. A mottled infiltrate was noted in the second and third interspaces on the left and a patchy infiltrate on the right. The findings in

the left upper lobe were thought to represent shrinkage, and radiologic diagnoses of "pneumonitis and shrinkage of the left upper lobe with progression due to tuberculosis or carcinoma" were made. The patient was begun on a regimen consisting of 300,000 units of penicillin intramuscularly every three hours and 0.5 gm. of streptomycin intramuscularly every six hours. His temperature response was dramatic after institution of this treatment, and he became afebrile within twenty-four hours.

Although he had been irrational frequently during his hospitalization, this symptom became progressively more severe after the episode of pneumococcal pneumonia. On the twenty-ninth hospital day he became obtunded and was found to have a marked nuchal rigidity and a positive Kernig sign. Except for abdominal distention there were no other new abnormalities on physical examination. Lumbar puncture was repeated and an initial pressure of 300 mm. of water and a final pressure of 150 mm. of water were noted. The spinal fluid contained 127 cells of which 85 per cent were lymphocytes and 15 per cent polymorphonuclear leukocytes. On standing, the fluid formed a cloudy yellow pellicle. The protein content was 278 mg. per cent, the sugar 0. Smears of the pellicle for bacteria and for acid-fast organisms were negative. Studies directed toward the isolation of cryptococcus were also negative. Despite the fact that numerous cultures and guinea pig inoculations had failed to substantiate the diagnosis of tuberculosis, the patient was given 2 gm. of streptomycin intramuscularly and 200 mg. of isonicotinic acid hydrazid by mouth daily. He also received 12,000,000 units of penicillin intramuscularly daily. After two days on this regimen lumbar puncture was repeated. The fluid contained 145 mg. per cent protein and no sugar. A smear revealed two polymorphonuclear leukocytes and ninety-six red blood cells, fifty-two of which were crenated. Routine cultures were sterile. Acid-fast stain of the pellicle which formed was again done and no organisms were found.

The patient continued to cough up foul-smelling purulent sputum from which, on two occasions, pneumococci were cultured but acid-fast organisms were never found. A biopsy of one of the subcutaneous nodules on the back was reported to show metastatic adenocarcinoma.

The patient remained comatose until the day of his death. Daily temperature elevations ranged from 37.5° to 39.6°C. On the thirty-seventh hospital day the patient suddenly became hypotensive and thereafter required nor-epinephrine intravenously for maintenance of normal blood pressure. His course was otherwise unchanged. On the forty-fifth hospital day the non-protein nitrogen was 23 mg. per cent, potassium 3.4 mEq./L., carbon dioxide combining power 31.8 mEq./L. and chloride 96 mEq./L. Despite continuation of nor-epinephrine the blood pressure fell precipitously, the patient had a clonic convulsion and he expired on the forty-fifth day, April 27, 1953.

CLINICAL DISCUSSION *

DR. W. BARRY WOOD, JR.: Before we begin the general discussion I am going to ask Mr. Nicolay to review the radiologic findings for us.

MR. WILLIAM E. NICOLAY: On the day after the patient entered the hospital a postero-anterior view of the chest was obtained. There were no demonstrable abnormalities of the soft tissues of the bony thorax and the heart size was normal. Examination of the lung fields revealed the right lobe to be more radiolucent than normal and there was elevation of the left hilum. As indicated in the protocol the streaking from the left hilum to the upper lobe on that side was present initially but it was not mentioned specifically in the original report. There was also some patchy infiltration and calcification. The lateral view showed an increase in the anteroposterior diameter of the chest but again the heart was normal in size. There was elongation and tortuosity of the thoracic aorta. In the lateral view the fibrosis mentioned previously could be demonstrated to extend mainly into the apical section of the left lobe. These findings were considered compatible with fibrosis and shrinking of the upper lobe of the left lung, and in spite of the fact that the patient had negative PPD skin tests and a number of negative sputa the diagnosis of tuberculosis would still be most likely although both histoplasmosis and coccidioidomycosis would have to be considered. Almost a month after he entered the hospital the patient had another chest film which was obtained at the time he had pneumococcal pneumonia. The fibrosis in the left upper lobe

* It should be noted that this conference differs from those usually published in the Journal in that the discussion was carried on by students from the senior class rather than by members of the faculty.

was the same as previously noted but there was a new infiltration considered to be compatible with acute pneumonitis.

Turning now to a consideration of some of the other x-rays, a few days after the patient entered the hospital intravenous pyelograms were obtained. The right kidney was of normal contour but on the left there was a bifid pelvis. In the area near the right side of the sacrum curvilinear calcification thought to represent calcium in the wall of the left common iliac artery or perhaps calcification of an aneurysm was noted. The psoas shadows were normal. The right half of the sacrum was the site of mottled osteoporosis, possibly due to an osteolytic process. Other films of the pelvis were obtained and aside from the osteolytic lesions noted no other abnormalities were demonstrated. Both femora and the iliac bones were normal.

The first myelogram was unsatisfactory but on the second examination there was narrowing of the interspaces between the third and fourth and fourth and fifth lumbar vertebrae and between the fifth lumbar and first sacral vertebrae. A distinct filling defect seen between the fourth and fifth lumbar vertebrae was considered to be compatible with herniation of the intervertebral disc. In the region of the first sacral segment there was a filling defect not thought to be due to a disc but rather to some other mass, presumably tumor. Taking all the radiologic findings into consideration it seemed most likely that the patient had carcinomatosis with tuberculosis an alternative choice, especially in view of the pulmonary findings.

DR. WOOD: Thank you very much, Mr. Nicolay. Dr. Wilson, do you want to add anything to Mr. Nicolay's description?

DR. HUGH M. WILSON: No, he covered the subject admirably.

DR. WOOD: This case presented a difficult diagnostic problem. Although the patient was in the hospital a number of weeks, a definitive diagnosis was not made prior to his death. Mr. Nathans, will you begin by considering the various diagnostic possibilities?

MR. DANIEL NATHANS: The pathologic report of the subcutaneous nodule makes it clear that the patient had metastatic adenocarcinoma. Further, I think that he almost certainly had meningeal carcinomatosis. To be less specific, the spinal fluid findings were compatible with subacute meningitis.

DR. WOOD: I presume you call it subacute because of the nature of the inflammatory process.

MR. NATHANS: Yes.

DR. WOOD: Before we proceed to a consideration of other causes of subacute meningitis, Mr. Langdon, would you care to suggest any other diagnoses?

MR. DAVID E. LANGDON: The history and findings were indicative of several genitourinary abnormalities, among them nodular hyperplasia of the prostate. The hard-fixed nodule noted on rectal examination directs attention to the prostate as the primary site of the malignant process.

MR. STUART WEISS: The patient probably suffered from a chronic urinary tract infection; he gave a history of long-standing obstruction and the urine contained white cells.

DR. WOOD: What sort of infection do you think he had?

MR. WEISS: I would suspect that he had both chronic prostatitis and urethritis. His renal function remained good so presumably the kidneys were not significantly involved.

DR. WOOD: Let us turn now to a consideration of the pulmonary disease. Evidently pneumococcal pneumonia developed while the patient was in the hospital. The acute infection responded well to therapy but there was residual scarring and fibrosis. Mr. Meredith, what do you expect the lungs to show pathologically?

MR. DONALD C. MEREDITH: Metastatic or primary carcinoma of the lung seem to me to be the two most likely possibilities. The combination of purulent sputum and hemoptyses suggest possibly lung abscess secondary to carcinoma.

DR. WOOD: Would you consider tuberculosis seriously?

MR. MEREDITH: No, I would not.

DR. WOOD: Does anyone wish to join the radiologists and support the diagnosis of tuberculosis?

MR. ANDREW MCCANSE: Despite the fact that three PPD skin tests were negative the radiologic findings seem more compatible with tuberculosis than with any other disease. Although negative PPD tests are rare in the presence of active tuberculosis, they may occur during the incubation period of the disease, in overwhelming infections or after complete healing.

DR. WOOD: Are there any other comments?

MR. DAVID L. GLOBUS: In any case it would be safe to assume that the left upper lobe will show a good deal of fibrosis.

MR. MCCANSE: The patient certainly also had emphysema as evidenced by the increase in the anteroposterior diameter of the chest, resonance to percussion and radiolucency.

DR. WOOD: It will be recalled that this patient had a lesion involving a large portion of the skin over his leg and back. Do you have any comments about this finding, Mr. McColl?

MR. HARRY A. MCCOLL, JR.: Only that it represented chronic dermatitis which I would be unable to define any more specifically than that. I doubt that it played any role in the terminal illness.

DR. WOOD: Major problems in this case were two in number, namely, the nature of the process in the subarachnoid space and the origin of the malignant tumor demonstrated in the skin metastasis. Let us deal first with the meningitis. The patient had a number of lumbar punctures and on several occasions the cerebrospinal fluid pressure was increased. There was a distinct elevation of the spinal fluid protein and a very low sugar content. The chlorides remained essentially normal and smears and cultures were negative for bacteria and fungi. What diagnostic possibilities occur to you, Mr. Grant?

MR. JOHN M. GRANT: Since it was definitely shown that metastatic carcinoma involved the skin, one should list first carcinomatosis of the leptomeninges which can give a clinical picture almost identical with aseptic menignitis.

DR. WOOD: Would it explain the absence of sugar in the cerebrospinal fluid?

MR. GRANT: Yes, in most cases of carcinomatosis of the leptomeninges, the spinal fluid sugar has been below 15 mg. per cent. In a recent article[1] cases are reported in which the findings were of that order.

DR. WOOD: You are referring to the article by Dr. Leonard Berg who graduated from this school and was an intern on the Ward Medical Service before joining the staff of the Neurological Institute in New York. The occurrence of low spinal fluid sugar in meningeal carcinomatosis is an important point, one not well recognized. We discussed this subject at some length when this patient was on the ward; there is only a limited amount of data in the literature but in the series published approximately 80 per cent of the patients with meningeal metastases did exhibit low spinal fluid sugar. What other causes for the spinal fluid changes should be mentioned?

MR. GRANT: Tuberculous meningitis certainly has to be considered. Pellicles formed in the spinal fluid on several occasions; and although they also occur in the presence of carcinomatosis, they are common in tuberculous meningitis. Against the latter diagnosis, of course, is the fact that the cultures and smears were repeatedly negative for acid-fast organisms.

DR. WOOD: Are there any other forms of infectious meningitis which should be listed?

MR. GRANT: Cryptococcal meningitis should be included.

DR. WOOD: Do you think that is a likely possibility, Mr. Merrims?

MR. THEODORE MERRIMS: It is said that the cryptococcal meningitis may closely mimic tuberculous meningitis.

DR. WOOD: How are torula organisms demonstrated in spinal fluid?

MR. LANGDON: The simplest technic is an India ink preparation. The ink does not penetrate the capsule of the organisms and they stand out well against the dark background.

DR. WOOD: Yes, that method is relatively easy and effective. It should be pointed out that repeated examinations may be necessary and a high index of suspicion is important. Mr. Merrims, can you add any other etiologic agents?

MR. MERRIMS: No. Most of the viral forms of meningitis can be excluded by virtue of the low spinal fluid sugar.

DR. WOOD: Does the spinal fluid sugar ever fall to 0 in viral meningitis, Mr. Vanderpearl?

MR. ROBERT H. VANDERPEARL: Not that I am aware of.

DR. WOOD: Would you agree, Dr. Harford?

DR. CARL G. HARFORD: Yes, I would.

DR. WOOD: In addition to carcinomatosis and chronic bacterial and fungus infections of the meninges, a low spinal fluid sugar is common in acute bacterial meningitis. What factors militate against that type of infection in this case?

MR. MERRIMS: Both the clinical course and the number and character of the cells were entirely out of keeping with any form of acute bacterial meningitis.

DR. WOOD: Are there any other suggestions?

MR. ARMIN C. HOFSOMMER, JR.: I would like to mention without much conviction the possibility of lymphopathia venereum.

DR. WOOD: Is meningitis due to that virus associated with a low spinal fluid sugar?

[1] BERG, L. Hypoglycorrhachia of non-infectious origin: diffuse meningeal neoplasia. *Neurology*, 3: 811, 1953.

MR. HOFSOMMER: In some cases it has been reported to be as low as 17 mg. per cent. I did not find any where the value was 0.

DR. WOOD: What other reasons led you to suggest lymphopathia?

MR. HOFSOMMER: First, the patient had significant symptoms referable to the rectum, e.g., painful defecation and bleeding. Second, he described what may well have been inguinal lymph node enlargement prior to his entry to the hospital. It is true that when he was examined here, the nodes were described as small and shotty and did not sound impressive.

DR. WOOD: Could the pulmonary lesions be explained on the basis of lymphopathia infection?

MR. HOFSOMMER: Lymphopathia venereum can produce pneumonitis but it would be stretching the point to assume that the patient's pulmonary findings were due to the virus.

DR. WOOD: Your suggestion is an interesting though probably not a very likely one. Are there any other comments?

MR. LANGDON: It is generally stated that in infectious meningitis, for example, tuberculous or torula, there is not only a high spinal fluid protein content but also an increased cell count. In this case there was consistent disassociation between the two, the protein having been higher than one would have expected within the slight increase in cells. This observation suggests that the patient did not have infectious meningitis. Further, if one calculates the Ayala index, the value is at the lower limit of normal and more in keeping with a space-occupying lesion than with infection.

DR. WOOD: I take it then that you favor carcinomatosis of the leptomeninges.

MR. LANGDON: Yes.

MR. VANDERPEARL: I support the diagnosis of tuberculosis but I do so on the basis of additional information not given in the protocol. The two spinal fluid chlorides values given were both 120 mEq./L., but in all actually four chloride determinations were performed. The two values not included here were, respectively, 100 mEq./L. and 114 mEq./L. My reason for favoring tuberculosis over carcinomatosis is that I have not been able to find a single case in the literature in which the spinal fluid chloride has reached so low a level in carcinomatosis. Another factor against the diagnosis of carcinomatosis are that only three cases have been reported in which the sugar was zero in the spinal fluid. Then, too, headache is common with carcinomatosis and the cranial nerves are frequently involved. Finally, pellicle formation is said to be rare in carcinomatosis.

DR. WOOD: What is the significance of pellicle formation?

MR. VANDERPEARL: It is due to the presence of a large amount of protein, particularly fibrin and fibrinogen, in the spinal fluid.

DR. WOOD: Do pellicles occur only in tuberculosis?

MR. VANDERPEARL: No, although they are characteristic of the fluid in tuberculosis meningitis, they are found in a number of other conditions, for example, in subarachnoid block.

DR. WOOD: Since pellicle formation reflects high spinal fluid protein content, it follows that in any condition in which there is a high spinal fluid protein a pellicle may form. For that reason, the presence of a pellicle does not represent a useful criterion in differential diagnosis. Why does the spinal fluid chloride fall?

MR. VANDERPEARL: That is a difficult question to answer because adequate information bearing on this point is not available. It has been postulated that as the protein in the spinal fluid increases there is a change in osmotic equilibrium and the chloride level falls. The level of the spinal fluid chloride has also been said to vary directly with the serum chloride.

DR. WOOD: Mr. Nathans, why does the spinal fluid sugar fall in bacterial and fungal meningitis?

MR. NATHANS: The fall has been attributed to metabolism of glucose by bacteria and/or leukocytes. In the study reported by Drs. Goldring and Harford, however, neither high cell count nor the presence of many bacteria adequately explained the low spinal fluid sugar.[2]

DR. WOOD: One must postulate, therefore, that there is a disturbance of secretion of spinal fluid in certain inflammatory conditions. Is there good evidence that the choroid plexus does have secretory function?

MR. GLOBUS: In a recent lecture Dr. Flexner of Baltimore presented evidence that it does.

DR. WOOD: Yes, his studies suggest that during the development of the embryo the choroid has active secretory function.

DR. ROBERT A. MOORE: Dr. Wood, I have a slide of the biopsy and would be glad to show it at this time.

[2] GOLDRING, S. and HARFORD, C. G. Effect of leucocytes and bacteria on glucose content of the cerebrospinal fluid in meningitis. *Proc. Soc. Exper. Biol. & Med.*, 75: 669, 1950.

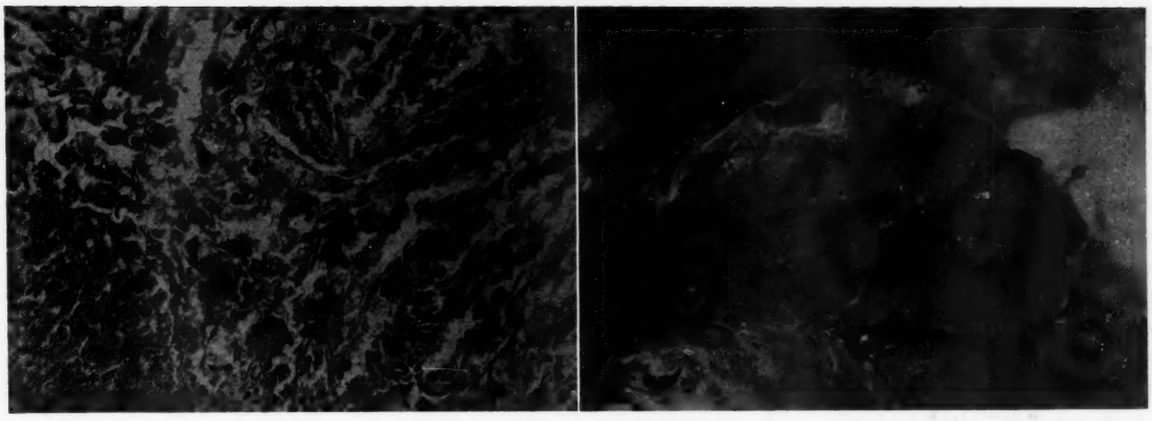

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FIG. 1. A section of the biopsy showing anaplastic polyhedral cells and necrosis.

FIG. 2. The apical segment of the left lung with areas of consolidation and sharply circumscribed cavities filled with purulent material.

DR. WOOD: That would be very helpful.

DR. R. A. MOORE: I can be objective about this section because I looked at it before I knew anything about the case. I will try to indicate my line of reasoning at the time I examined this specimen. Figure 1 is a characteristic section from the subcutaneous nodule and shows typical tumor cells throughout. The cells are polygonal with a fairly large chromatic nucleus and are arranged in sheets and very occasionally in acinous form which suggests that they have retained at least some capacity to form an alveolar pattern. There are no intercellular bridges or suggestion of pearls. If this is a squamous cell tumor, it has departed very far from its original pattern, and on the other hand no glandular pattern is very conspicuous. In essence, it is impossible to go much beyond the designation undifferentiated carcinoma. It is most unlikely that it is a carcinoma of the prostate, because carcinoma of the prostate rarely deviates this far from its glandular pattern. The degree of hemorrhage and necrosis is also atypical for a tumor of prostatic origin.

DR. WOOD: All of us who saw this patient in the hospital were worried about carcinoma of the prostate as the most likely primary tumor, but your comments, Dr. Moore, would lead us to look elsewhere. Mr. Hofsommer, you reminded us that this man has symptoms referable to the rectum. Do you want to support the diagnosis of adenocarcinoma of the rectum.

MR. HOFSOMMER: It certainly has to be considered. He did not have a significant degree of anemia but that's not necessarily present. The patient had no proctoscopic examination and no barium enema and therefore it would be difficult to be sure that he did not have a tumor somewhere in the lower gastrointestinal tract.

DR. WOOD: Actually, this patient was so ill during his hospitalization that several studies which would have been performed otherwise could not be undertaken. Had it been possible to do them, however, both proctoscopy and a barium enema might have been helpful. Are there any other studies which should have been done?

MR. HOFSOMMER: Cytologic examination of the sputum might have been helpful.

DR. WOOD: Yes, that should have been done. Anything else?

MR. NATHANS: One could have looked for malignant cells in the spinal fluid.

DR. WOOD: Have we ever successfully demonstrated cancer cells in the spinal fluid, Dr. Moore?

DR. CARL V. MOORE: No, I do not believe so.

DR. WOOD: I doubt that we have made many attempts to do so but it is a worthwhile procedure when indicated. Does anyone wish to suggest a different primary site for the tumor?

MR. WEISS: Carcinoma of the lung, which was mentioned earlier, is a reasonable possibility.

DR. WOOD: Mr. Globus, the fact that Dr. Robert Moore's opinion of the biopsy specimen is that it does not represent metastatic tumor from the prostate is important, is it not. Dr. Moore has been interested in diseases of the prostate gland for years and, as I indicated earlier, all of us would be influenced by his opinion. Were any of the laboratory findings against the diagnosis of carcinoma of the prostate?

MR. GLOBUS: The low acid phosphatase.

DR. WOOD: How valuable is that determination?

MR. GLOEUS: It is usually elevated when the tumor has metastasized outside the prostatic gland itself.

MR. MEREDITH: Gutman points out two situations in which metastases from prostatic carcinoma may not be associated with elevation of the acid phosphatase; first, if the metastases are of such long duration that the cells become depleted and no longer produce the enzyme, and second, in the presence of physiologic castration.

DR. WOOD: In summary, Dr. Moore, this patient clearly had some form of meningitis, the consensus being that it was due to adenocarcinoma involving the meninges. The primary site of the carcinoma is obscure but among the possibilities are the prostate, the colon or the lung.

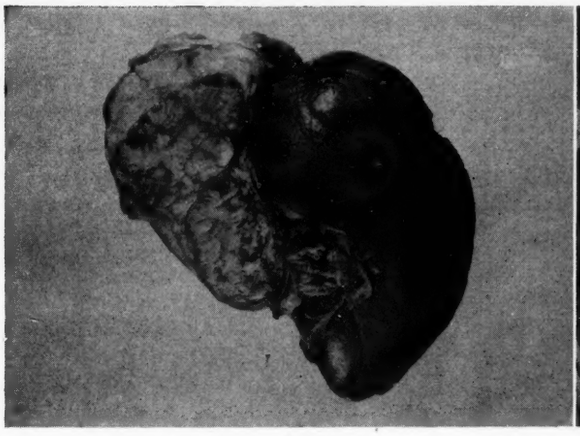
Clinical Diagnoses: Carcinoma of undetermined site with metastases to the skin and leptomeninges; chronic prostatitis and urethritis; pulmonary fibrosis, ? tuberculous.

PATHOLOGIC DISCUSSION

DR. CHARLES R. UNDERWOOD: The right lung was increased in weight to 1,280 gm. and the left to 960 gm. Firm adhesions bound the apices of both lungs to the chest wall. The parenchyma was quite firm and throughout all lobes there were many small yellow-white areas of consolidation. In the upper lobes there were several small sharply circumscribed cavities 2 to 4 mm. in diameter which contained mucopurulent material. (Fig. 2.) This process involved all lobes of the lungs and was more advanced in the upper lobe of the left lung. In the right perirenal space a large tumor mass 14 by 8 by 6 cm. in its diameters lay over the superior pole of the right kidney. (Fig. 3.) It was abutting against the renal capsule but there was no gross evidence of invasion of the parenchyma of the kidney. Cut section had a somewhat nodular appearance. In several areas there was hemorrhage into the tumor; in other areas there were foci of yellow necrotic degeneration. The right adrenal vein was invaded as is shown in Figure 4 and a fungating mass of tumor extended into the vena cava. The left adrenal was enlarged and contained several nodules of tumor like that which replaced the right adrenal. The mucosa of both renal pelves and of the urinary bladder was congested and contained many petechiae. There was a bifid renal pelvis on the left with a bifid ureter that joined at the rim of the pelvis. The double ureters were dilated above the point of junction. The lymph nodes in the region of the periaortic chain, about the pancreas and up the aorta as high as the diaphragm were enlarged to as much as 2 cm. in diameter. The tissue filling these large lymph nodes was yellowish white and resembled that in the right adrenal. The liver weighted 2,060 gm. and contained many 1 to 2 cm. nodules of gray-yellow tumor tissue.

The vertebral canal was opened and the cord was removed. In the region of the lumbar sac in the epidural space there was a firm cast of yellow tumor tissue which completely encased and compressed the dura of the cauda equina. The tumor extended laterally along the nerve roots. Infiltration of the subarachnoid space could not be grossly recognized. The vertebral bodies were soft and osteoporotic but there was no evidence of metastases within the bone or the intervertebral discs. The prostate was moderately enlarged and contained whorled white nodules in the lateral lobes. There were no lesions within or enroaching upon the intestine.

DR. R. A. MOORE: It is clear from the description of the gross appearance of the organs and the clinical signs and symptoms that we have three categories of disease with which to deal in this case. From the gross examination they appear unrelated. First, this man had a malignant tumor primary in the right adrenal gland with invasion of the adrenal vein, many metastases, and involvement of the epidural space of the lower spinal cord with compression. Second, there was a disease of the lung characterized by the formation throughout all lobes, most particularly in the left upper lobe, of small rather sharply circumscribed spherical masses filled with yellow friable or purulent material. The parenchyma between was consolidated and grossly organized. This picture is fairly typical of chronic bronchiolectasis and bronchiolitis in which small bronchi, particularly at the periphery of the lung, are dilated and filled with purulent material and there is gradual erosion of the wall and dilatation. The lesions take on the appearance of abscesses except they are always sharply outlined in contrast to the irregular outline of a true abscess. Third, there was disease of the



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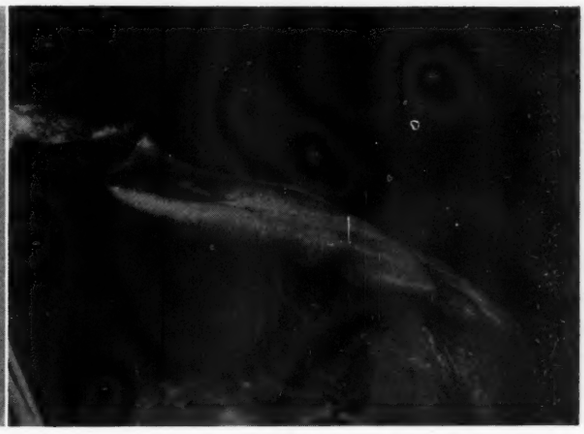

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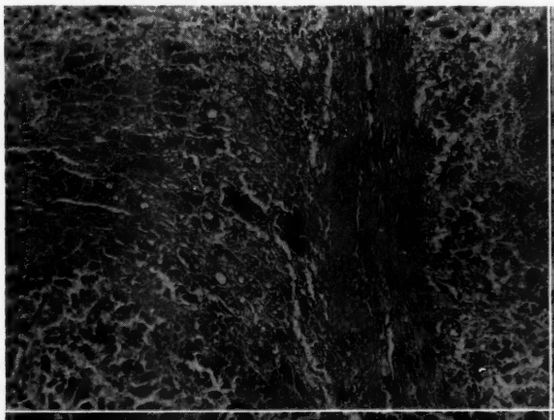
FIG. 3. The right kidney with the overlying mass of nodular gray-yellow tumor that replaces the right adrenal gland. There are extensive areas of necrosis and some hemorrhage in this tumor.

FIG. 4. The inferior vena cava with a thrombus of tumor extending from the ostium of the right adrenal vein.



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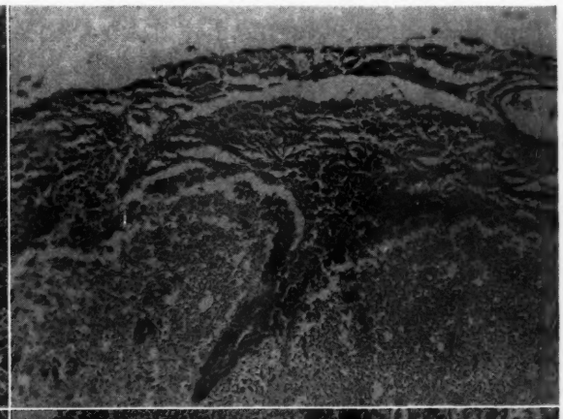

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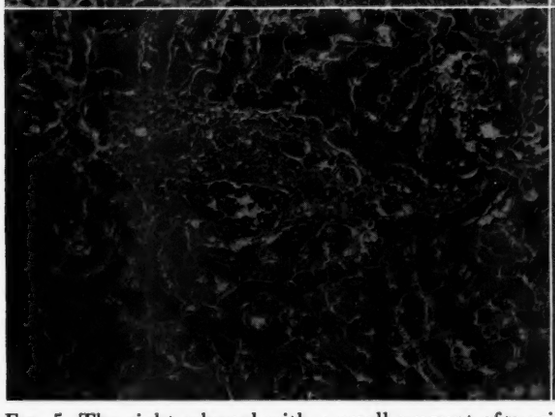

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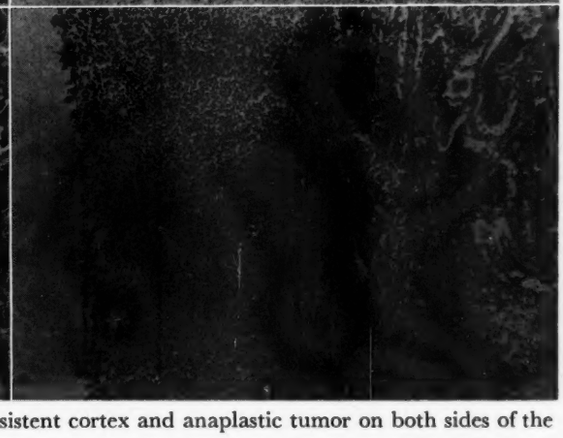

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FIG. 5. The right adrenal with a small amount of persistent cortex and anaplastic tumor on both sides of the capsule.

FIG. 6. The sacral spinal cord showing infiltration of the subarachnoid space and Virchow-Robin spaces by metastatic tumor.

FIG. 7. Metastatic tumor within the veins in the portal spaces of the liver and as isolated cells within the hepatic sinusoids. This type of metastasis was present diffusely throughout the liver.

FIG. 8. A section of a small cavity in the apex of the left lung. The wall shows acute and subacute inflammation with destruction of the bronchiolar wall and only slight organization of the surrounding tissue of the lung. This type of reaction is typical of that found in bronchiolectasis.

prostate which so far as we can determine grossly and microscopically was nodular hyperplasia with slight chronic inflammation but without evidence of carcinoma within the prostate itself. The gastrointestinal tract was relatively normal and there was no lesion as an apparent cause of the bleeding.

Microscopic sections throw little additional light on these conclusions. Figure 5 illustrates a section from the tumor in the adrenal in which there are a few adrenal cells remaining and the same neoplastic cell seen in the surgical specimen. I think it is quite clear from its nature that this is a malignant tumor derived from the cells of the cortex of the adrenal. Figure 6 is a section of the sacral spinal cord. As was predicted in the discussion, this represents carcinomatosis of the meninges, particularly in the lower spinal cord, with the cord and nerve roots encased in tumor cells that invade along the Virchow-Robin spaces right into the substance of the cord. The grossly recognized epidural metastases were probably responsible for the changes in the myelograms, and pressure on the spinal roots, and the leptomeningeal carcinomatosis undoubtedly added to the signs and symptoms related to the central nervous system.

Figure 7 is a section of the liver showing rather remarkable microscopic permeation of the portal veins. Most of the portal veins have these thrombi of tumor cells almost or completely filling them. Occasional tumor cells are also present in the sinusoids. Figure 8 shows a section of the wall of one of the cavities in the lung. There is acute and subacute inflammation with destruction of the wall of the bronchiole within which the inflammation arose but with very little organization of the surrounding pulmonary tissue in this section. This finding is typical of bronchiolectasis.

In summary, this patient had a primary carcinoma of the right adrenal with invasion of the adrenal vein and metastases in the liver, left adrenal and periaortic lymph nodes. The tumor invaded posteriorly into the vertebral canal and encased the lumbosacral dural sac, giving rise to carcinomatosis of the leptomeninges in this region; there was no evidence of involvement of the brain or of the upper portion of the spinal cord. As a second and unrelated primary disease there was chronic purulent inflammation in the lungs with bronchiolectasis, small abscesses and organized pneumonia. In the genitourinary system nodular hyperplasia of the prostate and definite cystitis were found. The gastrointestinal signs and symptoms could not be related to any lesion present at autopsy.

Final Anatomic Diagnoses: Partially organized pneumonia in all lobes of the lungs with abscesses in the upper lobes and lower lobe of the left lung; bronchiolectasis in the apicodorsal segment of the upper lobe of the right lung; carcinoma of the right adrenal gland with invasion of the superior adrenal vein and inferior vena cava; metastatic carcinoma in the epidural, subdural and subarachnoid space of the spinal cord and cauda equina, periaortic, mesenteric, peripancreatic and super mediastinal lymph nodes, liver, left adrenal, right kidney and subcutaneous tissue in the area of the xiphoid; nodular hyperplasia of the prostate, moderate; acute cystitis.

Acknowledgment: Illustrations were made by the Department of Illustrations, Washington University School of Medicine.

Case Report

Fatal Hemorrhage from Esophageal Varices*

Due to Malformations and Congenital Stenoses in the Portal Venous System

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PORTAL hypertension with congestive splenomegaly is not uncommon. Cirrhosis of the liver, cavernous transformation of the portal veins, varices or acquired stricture are frequently responsible. In a few instances congenital strictures of the portal vein have been reported. Other cases have been reported in which surgical exploration failed to demonstrate a cause for the hypertension.

The case to be presented appears to have resulted from a bizarre but readily explainable combination of embryologic anomalies of the portal system. This patient has been included in two previously reported studies as an example of an instance of portal hypertension for which the etiology could not be established clinically or at operation.[1,2]†

The authors were unable to find a report of a precisely similar case but the embryologic features of the case suggest the possibility of similar occurrences. In addition, the clinical implications of the problem are of considerable interest.

CASE REPORT

The patient was first admitted in August, 1938, to the Presbyterian Hospital in New York City because of a history of previous gastrointestinal hemorrhage. She was then thirteen years old. The spleen was palpable 5 cm. below the left costal margin. The liver was palpable 2 cm. below the right costal margin.

Laboratory findings were as follows: Kline test, negative; urinalysis, negative. Preoperative blood findings: hemoglobin 12.9 gm./100 ml.; red blood count, 5,500,000/cu.mm.; white blood count, 6,200/cu.mm.; platelet count, 135,00/cu.mm.; reticulocyte count, 0.9 per cent. Laboratory tests showed no evidence of liver dysfunction.

A liver biopsy and splenectomy were performed. At operation the pressure was 330 mm. of water in the splenic vein and 55 mm. of water in the cephalic vein. There were a few adhesions about the upper pole of the spleen. The spleen was described as "four times normal size." Pathologic examination revealed "fibrosis of the spleen" and normal liver. The patient received intravenous heparin for six days and had an uneventful postoperative course. Postoperative blood count showed no change except for an increase in blood platelets to 296,000/cu.mm. After discharge she was followed in the outpatient department until 1947 during which time she had several minor episodes of hematemesis and intermittent tarry stools.

The patient was admitted to the Stamford Hospital on August 10, 1949, with severe hematemesis and transient loss of consciousness. She was three months pregnant at the time.

The patient appeared to be in shock. The blood pressure was 90/50 mm. Hg, pulse rate 112/minute, and respiration 24/minute. The abdomen was soft with slight rigidity in the epigastrium. The liver was palpable two fingerbreadths below the right costal margin. No spleen was palpable. There was a mass in the abdomen consistent with a three to four month pregnancy.

The laboratory data were as follows: red blood count, 3,600,000/cu.mm.; hemoglobin, 10.3 gm./100 ml.; hematocrit, 28 per cent; white blood count, 18,600/cu.mm.; 79 per cent neutrophils of which 2 were band forms, 18 per cent lymphocytes, 1 per cent monocytes. The red blood cells showed marked aniso-

† In reference No. 1, this case is listed as B.S. under Table I, "Banti-obstructive factor undetermined." The case is similarly designated in reference No. 2.

* From The Department of Laboratories, Stamford Hospital, Stamford, Conn.

cytosis, slight polychromasia and moderate hypochromia. A repeat blood count was done on the third hospital day. The red blood count was 2,160,00/cu.mm.; hemoglobin, 7.3 gm./100 ml.; hematocrit, 19 per cent; white blood count, 22,500/cu.mm.; neutrophils 82 per cent of which 2 were band forms, lymphocytes 16 per cent. Urinalysis showed occasional red blood cells and 10 to 12 white blood cells per high power field. At the same time the blood glucose was 177 mg./100 ml.; prothrombin time, 18 seconds (control 18 seconds); total protein, 4.9 gm./100 ml.; albumin, 3.0 gm./100 ml.; globulin, 1.9 gm./100 ml.; clot retraction time, three hours; CO_2 content, 38 mEq./L.; sodium, 150 mEq./L.; potassium, 4.9 mEq./L.

During the course of her illness, she received nine transfusions of blood Type A, Rh positive (CDE), Hr positive (c).

During the first twenty-four hours her bleeding was apparently controlled by tamponade of the lower esophagus with an air-filled balloon attached to a Levine tube. On the second hospital day she vomited the tube along with 300 ml. of blood. On the third day the patient began to have vaginal bleeding which terminated rapidly in a complete abortion. Following this the patient became completely comatose. Peripheral veins were collapsed and it was necessary to use femoral veins for blood transfusions. Her condition grew progressively worse and she expired on the fourth hospital day.

At postmortem examination the lungs showed marked edema and areas of subpleural hemorrhage. Microscopic examination revealed acute purulent bronchitis and bronchopneumonia as well as aspiration of blood and epithelial débris. The heart showed a few focal hemorrhages on the epicardial surface but no other changes.

The esophagus showed extreme edema of the mucosa and dilatation of the lower third. In this region there were a number of large varices. At least two of these communicated with the lumen of the esophagus through points of ulceration. There was, however, no demonstrable clot or other evidence of recent bleeding. The stomach and small intestine were essentially normal. The colon was filled with tarry feces; there was no evidence of bleeding from the colon itself. The liver showed no abnormalities on either gross or microscopic examination. The uterus was large and boggy. It contained blood clot and decidual tissue. The right ovary contained a corpus luteum of pregnancy.

The spleen had been removed but an accessory spleen measuring 4 cm. in diameter and weighing 6 gm. was found at the tail of the pancreas. Microscopic examination showed focal hemorrhagic necrosis, pulp hyperplasia and focal hematopoesis.

The splenic vein was found to terminate on the tail of the pancreas in a small knot of black silk contained in a cavity measuring 1 cm. in diameter which was filled with a creamy yellow fluid. This fluid showed no bacteria on stained smear but did contain crystals of foreign material.

There were two separate portal veins arising from the porta hepatis. (Fig. 1.) These passed over the anterior surface of the second and third parts of the duodenum and the head of the pancreas and were joined together in an anastomotic loop at the level of the lower border of the duodenum. The right portal vein (portal vein II) drained the cystic vein and ended in several branches buried in the areolar tissue of the porta hepatis. The left portal vein (portal vein I) ended in two branches, the right and left intrahepatic portal veins to respective lobes of liver. The extrahepatic portal veins passed posteriorly to the cystic duct and anteriorly to the hepatic ducts and common bile duct.

The splenic vein originated as previously described and passed along the posterior superior border of the pancreas and then looped anteriorly and downward over the anterior surface of the head of the pancreas to join the left portal vein at a point 1 or 2 cm. above the anastomotic loop. Just before this junction the splenic vein was joined by a markedly dilated coronary vein. The orifice at the junction of the splenic and portal veins was reduced to a slit measuring 2 by 1 mm.

There was no evidence of any mesenteric vein joining the system in its usual position. However, a hugely dilated mesenteric vein was found to join the right portal vein (portal vein II) at a point 1 to 2 cm. above the lowermost portion of the anastomotic loop. Its orifice at this junction was slit-like and measured 1 by 3 mm.

The splenic, mesenteric and coronary veins were uniformly dilated, thickened and hypertrophied. The vessels were smoothly lined with no evidence of nodularity, thrombosis or cavernous change. Microscopic examination showed phlebofibrosis and an increase in the thickness of the muscular coat, with some trabeculation of the wall. (Figure 1 illustrates the gross findings.)

There were no abnormalities in the biliary

duct system or in the hepatic arterial system. The cystic artery passed behind the two portal veins so that the veins lay between the cystic duct and the hepatic artery. The hepatic, mesenteric and splenic arteries originated normally. The ligamentum teres ended in the porta hepatis near the right portal vein (portal vein II).

It was concluded from postmortem findings that the patient died of hemorrhagic shock, following hemorrhage from ruptured esophageal varices. The varices resulted from portal hypertension due to congenital strictures and anomalous positions of the veins of the portal system.

In seeking an explanation for the analomalies responsible for death a consideration of the normal development is helpful. In the normal process of development in the 4 mm. embryo the vitelline veins enter through the yolk stalk running cephalad along the side of the foregut to

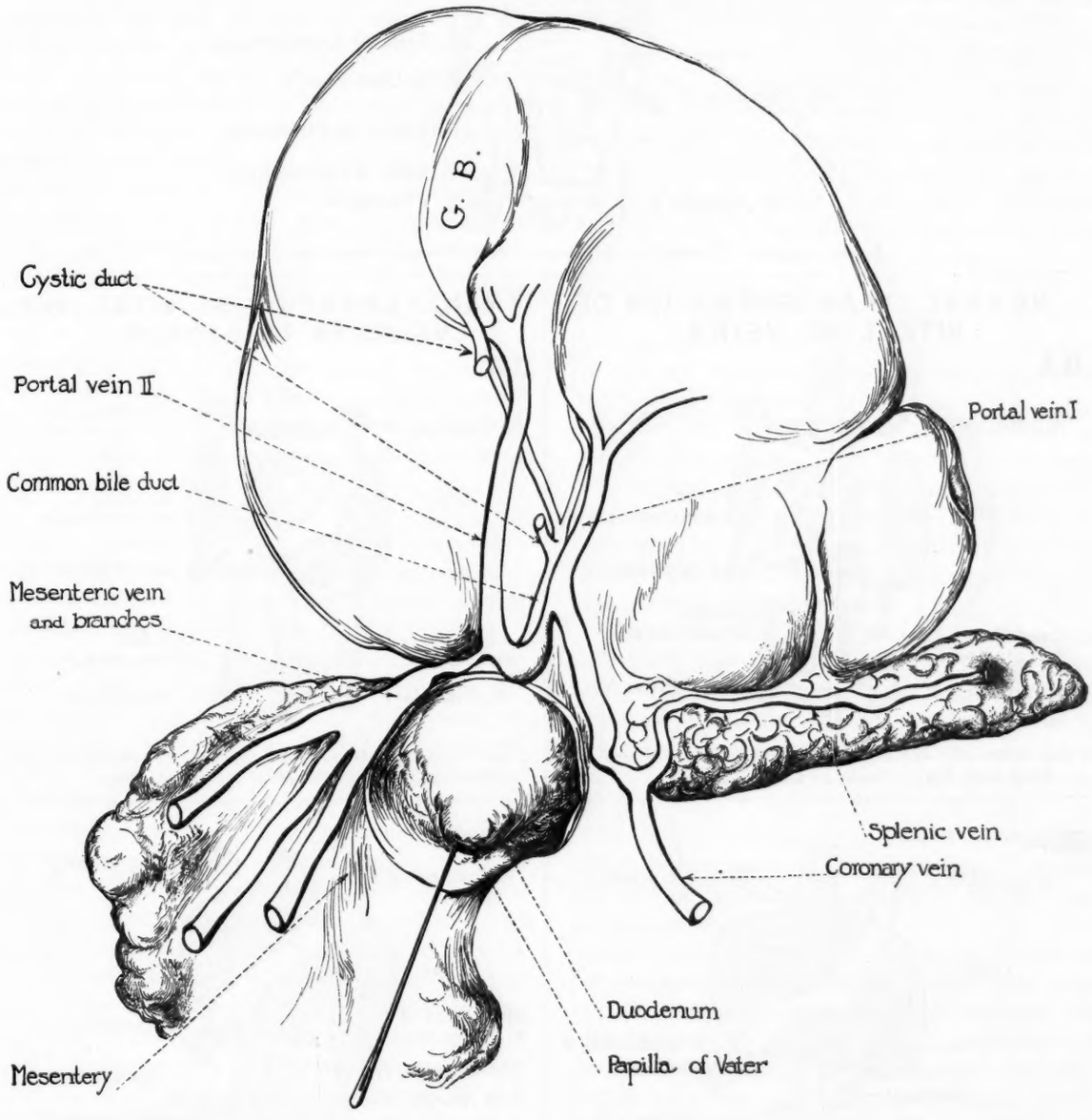

FIG. 1. Partially schematic drawing from a Kodachrome transparency. The positions of the organs have been modified to facilitate demonstration of the veins and branches. In particular, the body of the pancreas has been rotated forward to expose its posterosuperior surface.

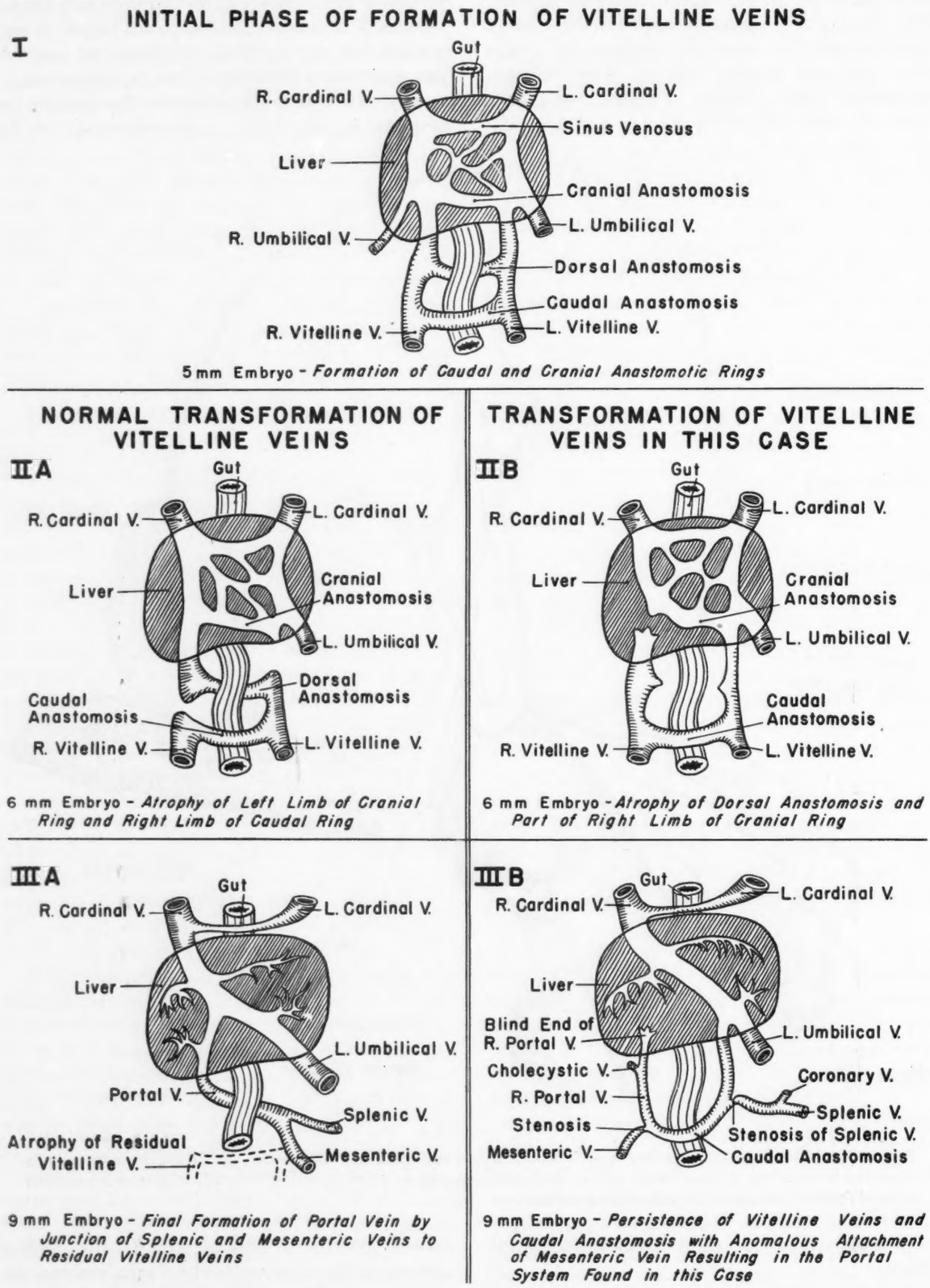

FIG. 2. Schema of important steps in the development of the normal portal system and the postulated deviations in this case. (Adapted from Arey's "Developmental Anatomy.")

enter the sinus venosus, through the septum transversum. At the same time the liver buds begin to develop in the septum transversum with a mutual intergrowth between the hepatic cords and the vitelline circulation. (Fig. 2.) By this process the vitelline veins are converted into three portions: the distal portion which eventually becomes the portal vein, the intermediate portion which remains as the sinusoids of the liver and also develops into the ductus venosus and the proximal portion which becomes the hepatic vein. The distal segments of the vitelline veins next form cross anastomoses around the foregut: a cranial loop within the liver and ventral to the gut, a middle loop dorsal to the gut and a caudal loop ventral to the gut. These together form a cranial and a caudal anastomatic ring about the gut. (Fig. 2I.)

Next there is atrophy of the left limb of the cranial ring and of the right limb of the caudal ring. (Fig. 2II A.) In addition the major part of the caudal ring also disappears, being replaced by the newly formed splenic and mesenteric veins.

The portal vein thus consists of the right limb of the cranial ring, the middle transverse anastomosis, and the junction of the splenic and superior mesenteric veins just caudal to the transverse anastomosis. (Fig. 2III A.)

The anatomic conditions found in this case can be explained by assuming that the caudal and cranial rings persisted except for atrophy of the middle transverse anastomosis, thus forming both a right and left portal vein lying anterior to the duodenum. (Fig. 2II B.)

The splenic vein joined at the usual point but the superior mesenteric vein made its junction to the right portal vein. (Fig. 2III B.) The stenosis at the points of junction of the splenic vein and of the superior mesenteric vein to the portal veins are probably the result of defective development since these points of constriction show no evidence in themselves of recent or old thrombosis or inflammation. For this reason it appears that these zones of constriction are congenital and not acquired. The thickening of the veins is confined to those branches distal to sites of stenosis and is therefore due to phlebofibrosis and muscular hypertrophy probably resulting from hypertension in the venous system.

COMMENT

Work in recent years has established the importance of portal hypertension as the common etiologic factor in so-called "Banti's syndrome" or congestive splenomegaly.[3–9] In many cases cirrhosis of the liver has been the obstructive factor. In others, extrahepatic obstruction of the portal vein by different lesions such as thrombosis and various malformations have been demonstrated. In a remaining large group no readily apparent obstructive factor has been found.

Recent advances in surgical treatment of congestive splenomegaly have demonstrated the value of various shunt operations as being in general more valuable than simple splenectomy except in cases in which the obstructive factor is confined to the splenic vein alone.[10–13] Proper selection of the operative procedure depends upon careful exploration and evaluation of the particular abnormality of the portal system in each case. This is often technically difficult under the conditions of operation, which may explain the failure to demonstrate extrahepatic portal obstruction in many of the cases of portal hypertension without cirrohsis. Operations to perform portacaval shunts have revealed many more of these extrahepatic obstructive anomalies. For the most part these conditions have only been mentioned in passing without complete descriptions as incidental to reviews of surgical case series or technic reports.

Many anomalies of the portal vein have been described most of which suggest that they are the result of the development of collateral circulation or recanalization of thrombosis, as in cavernous transformation of portal veins, or of acquired constrictions and varicosities at the porta hepatis.[14–18]

Some cases have been thought to represent congenital stenosis of the portal vein.[19–21] The absence of stigmata of acquired change supported this contention.

Two cases reported by F. Stengel[22] were of preduodenal position of the portal vein with normal position of stomach and duodenum. He also discussed a few previously reported cases of preduodenal position of the portal vein associated with *situs inversus* or malrotation of the stomach and small intestine. The various possible consequences of congenital variations in the development of the vitelline veins were discussed and schematically presented. None of these cases or those of Stengel appear to correspond to this case.

Certain clinical implications appear to follow from the anatomic findings. The first of these is that it is apparent that splenectomy could be

expected to be of no value. The second is that a shunt operation suitable in this case would offer formidable surgical problems.

Some consideration must be given to the possible effect of pregnancy as a precipitating cause of the hemorrhage. While it appears possible that there might be a relationship between pregnancy and the fatal episode, there is nothing which permits an opinion to be reached from the purely anatomic data.

SUMMARY AND CONCLUSION

1. A case of anomalous development of the portal system is presented which includes variations in the positions of the veins as well as multiple strictures, apparently congenital in origin.

2. An explanation of the anomalies on embryologic grounds is postulated.

3. The rarity and certain clinical implications of this condition are discussed.

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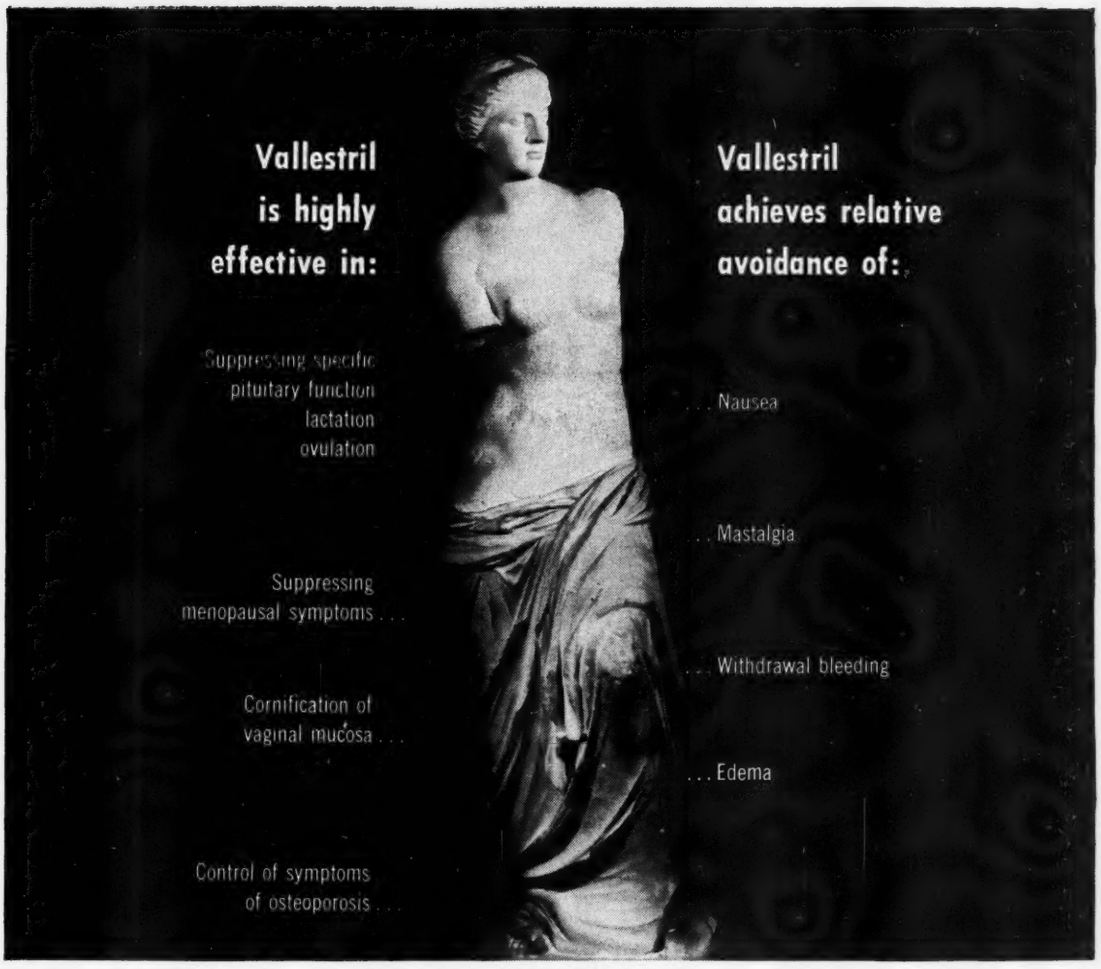

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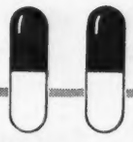

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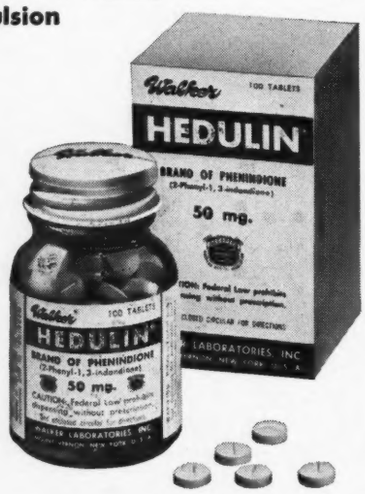

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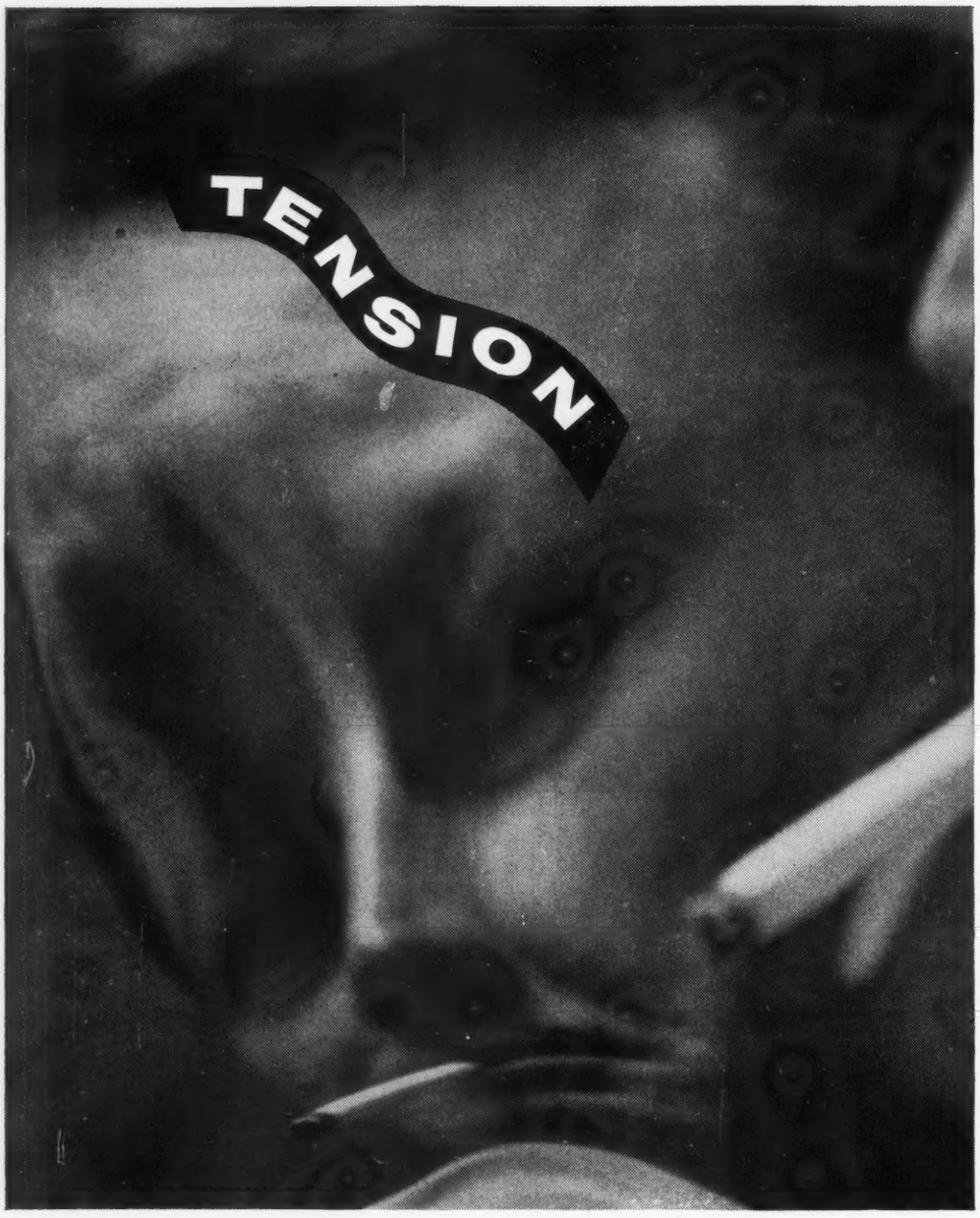

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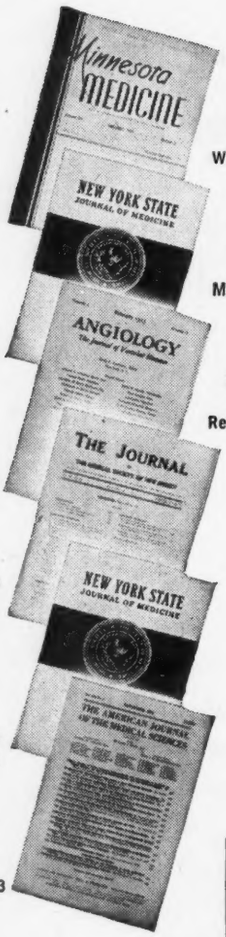

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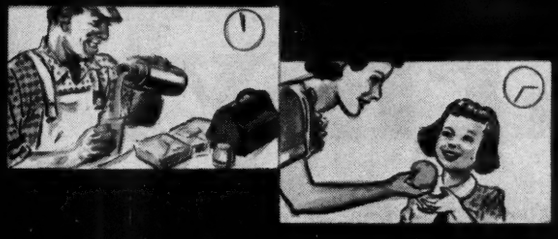

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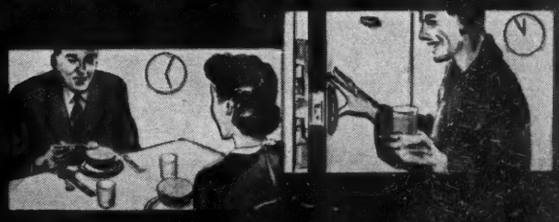

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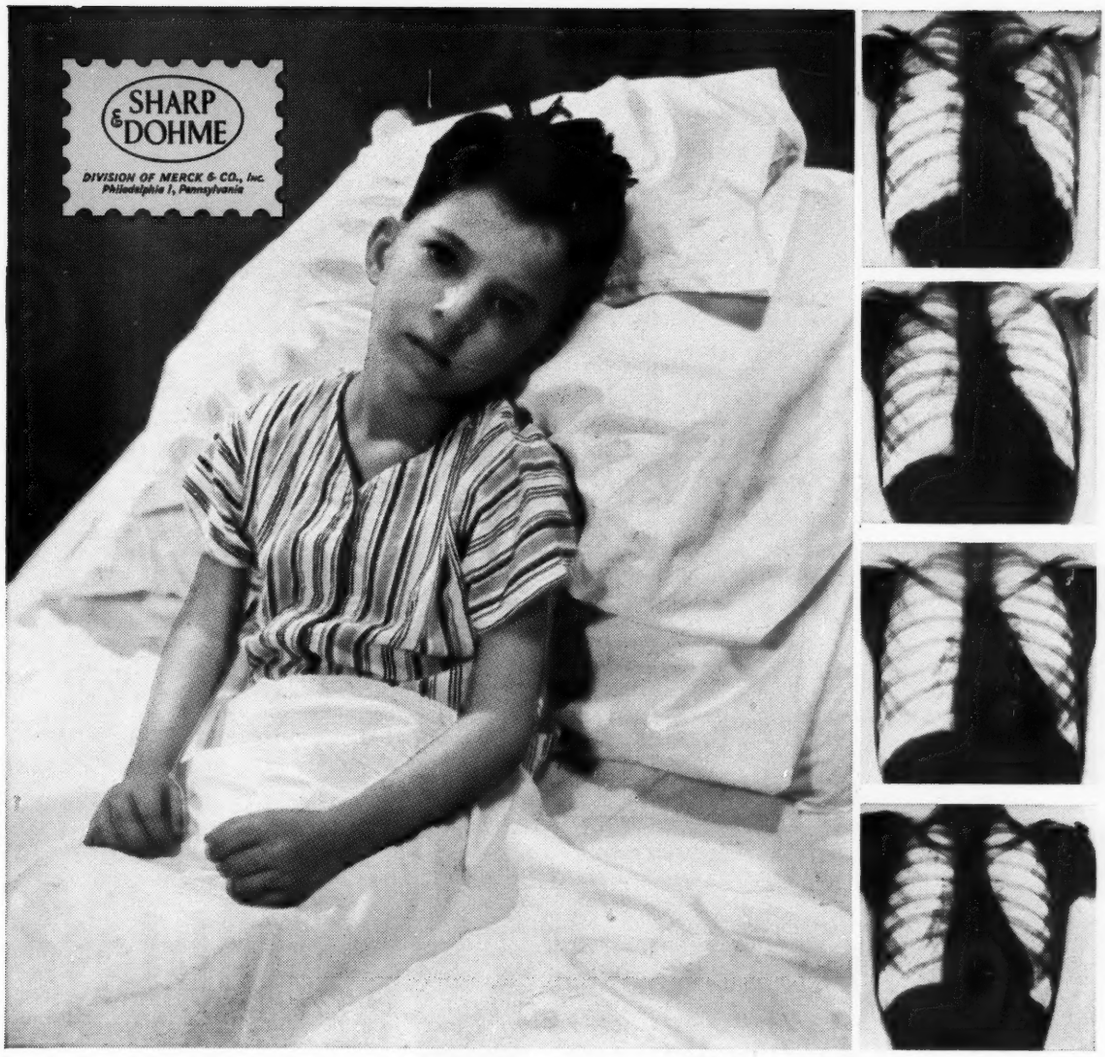

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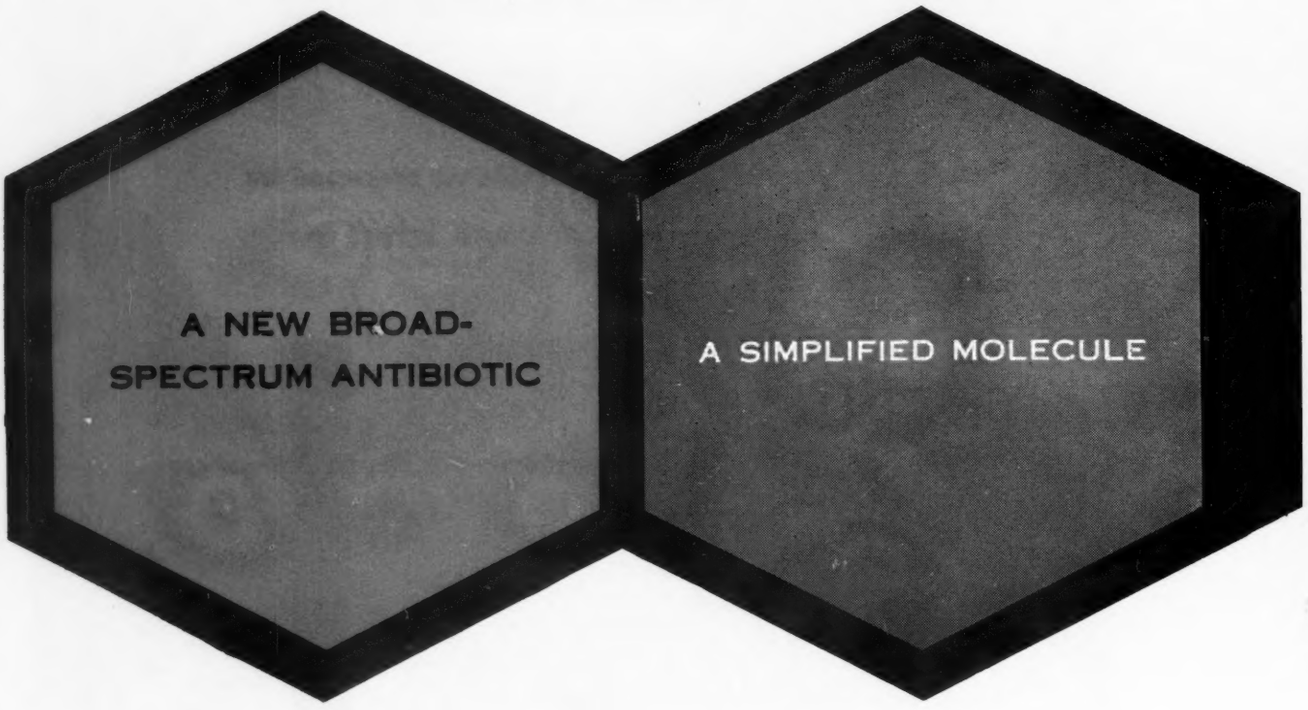

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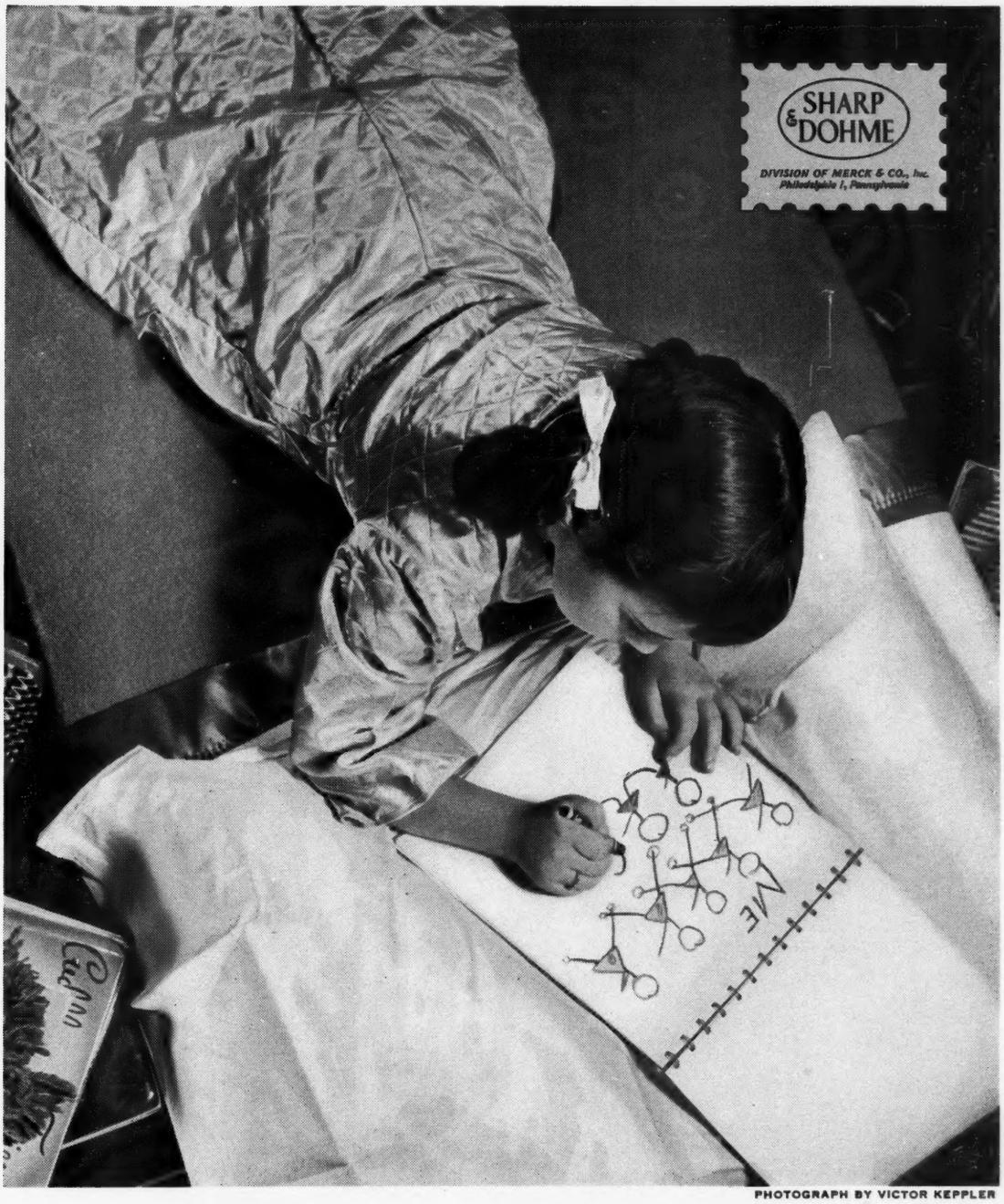

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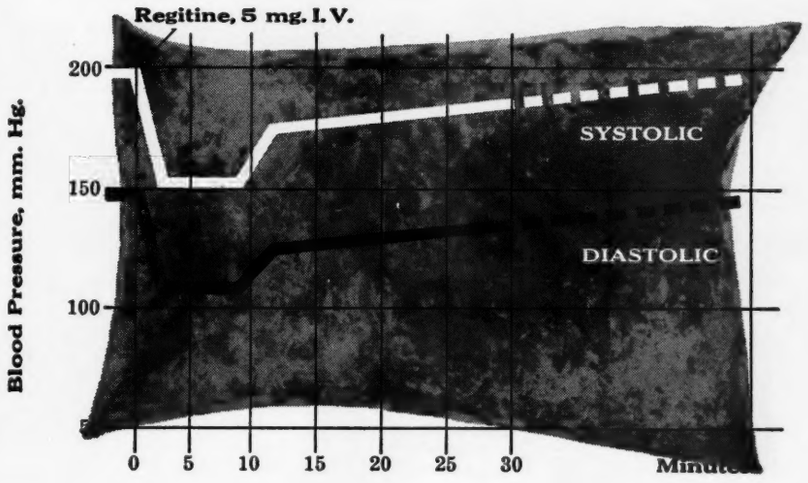

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Quick Information: Available in 0.5 *Gm.* tablets. *Dosage:* 1 to 4 tablets daily. *Contraindication:* renal impairment.

References: 1. Indust. Med. 22:311, 1953. **2.** J.A.M.A. 149:1188, 1952. **3.** Bull. Vancouver M.A. 29:306, 1953.

for the obese patient . . .

genuine Obedrin
obtainable
only on
prescription
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tablets are
monogramed
for your
assurance of
quality

Obedrin

Semoxydrine HCl	5 mg.
(Methamphetamine HCl)	
Pentobarbital	20 mg.
Ascorbic Acid	100 mg.
Thiamine HCl	0.5 mg.
Riboflavin	1 mg.
Niacin	5 mg.

massengill

For every patient
with clearcut menopausal
symptoms such as **hot flushes,**
there's another patient with symptoms less clearly defined
yet just as distressing . . . headaches,
insomnia, mental and physical fatigue.
Her symptoms may also be indicative of declining ovarian function, and occur
several years before, and even long after, menstruation ceases.
This patient, too, may be expected to **benefit** from "Premarin" therapy.

"PREMARIN"® is a complete equine estrogen-complex.
It not only produces prompt symptomatic relief, but also imparts
a distinctive **"sense of well-being"**
highly gratifying to the patient. It is tasteless and odorless.
"Premarin," estrogenic substances (water-soluble),
also known as conjugated estrogens
(equine), is supplied in tablet
and liquid form.

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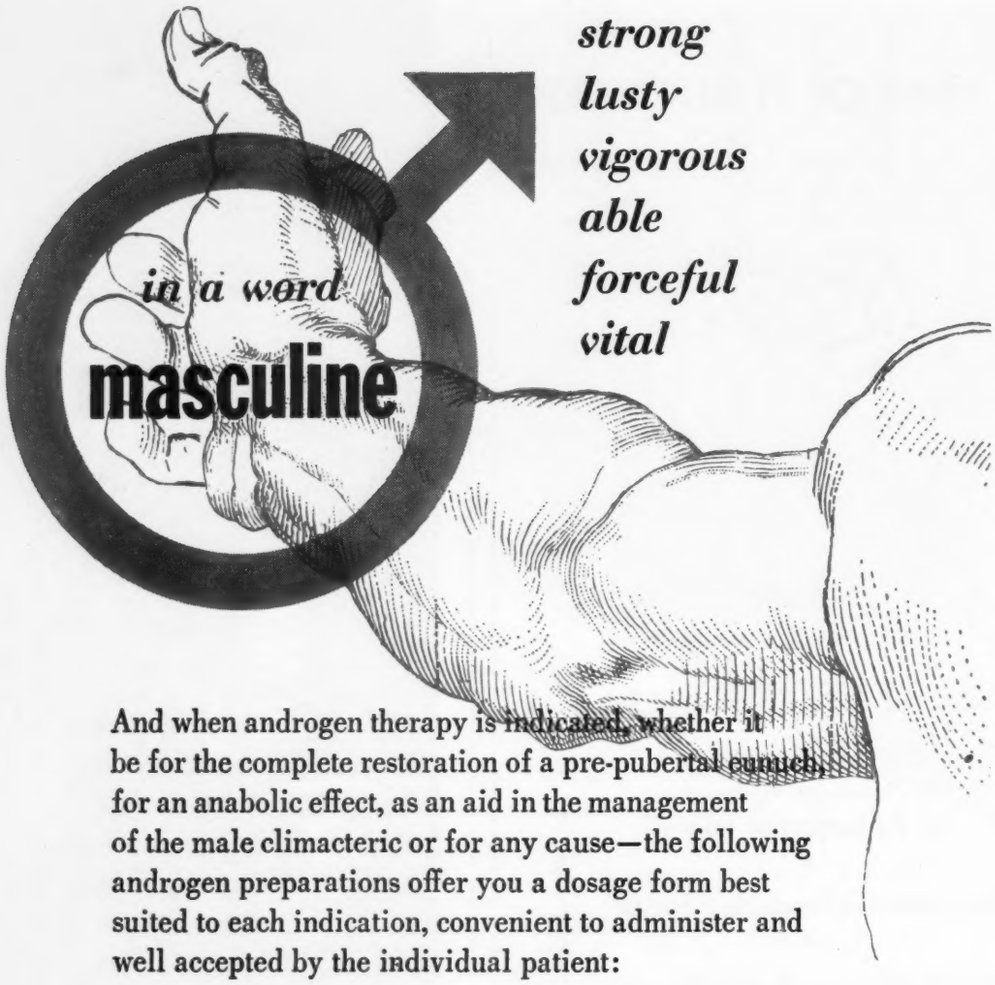
Ayerst

New York, N. Y. · Montreal, Canada



virile
strong
lusty
vigorous
able
forceful
vital

And when androgen therapy is indicated, whether it be for the complete restoration of a pre-pubertal eunuch, for an anabolic effect, as an aid in the management of the male climacteric or for any cause—the following androgen preparations offer you a dosage form best suited to each indication, convenient to administer and well accepted by the individual patient:

SYNANDROL* brand of testosterone propionate in sesame oil: 25 mg., 50 mg. and 100 mg./cc. in 10 cc. multiple-dose vials and in single-dose Steraject® disposable cartridges.

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SYNANDROTABS* brand of methyltestosterone tablets, for oral use: 10 mg. and 25 mg., bottles of 25 and 100.

SYNANDRETS* brand of testosterone transmucosal tablets, for absorption by the transmucosal route: 10 mg., bottles of 25 and 100; 25 mg., bottles of 25.

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*TRADEMARK

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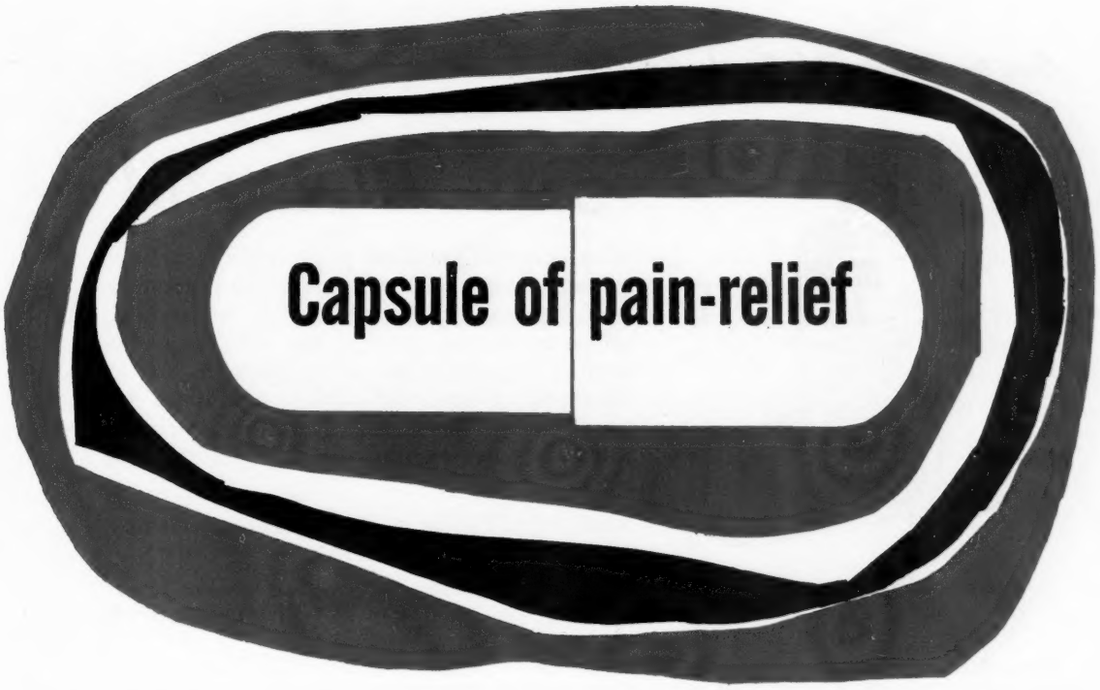


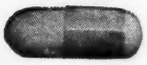
effective pain control
plus mild sedation

'Codempiral'®

Convenient dosage—two strengths

no. 2 Each capsule contains:

Codeine Phosphate	gr. ¼
Phenobarbital	gr. ¼
Acetophenetidin	gr. 2½
Aspirin	gr. 3½



no. 3 Each capsule contains:
Codeine Phosphate gr. ½
plus the other
ingredients listed above

Both strengths available in bottles of 100

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BURROUGHS WELLCOME & CO. (U.S.A.) INC., Tuckahoe 7, New York

For the Aged and Senile Patient

ORAL *Metrazol*

— to help the geriatric patient with early or advanced signs of mental confusion attain a more optimistic outlook on life, to be more cooperative and alert, often with improvement in appetite and sleep pattern.
Metrazol, a centrally acting stimulant, increases respiratory and circulatory efficiency without over-excitation or hypertensive effect.

Dose: 1½ to 3 grains, 1 or 2 teaspoonfuls Liquidum, or the tablets, every three or four hours.

Metrazol tablets, 1½ grs. (100 mg.) each. Metrazol Liquidum, a wine-like flavored 15 per cent alcoholic elixir containing 100 mg. Metrazol and 1 mg. thiamine HCl per teaspoonful.

Metrazol®, brand of pentylenetetrazol, a product of E. Bilhuber, Inc.

BILHUBER-KNOLL CORP. *distributor* — **ORANGE NEW JERSEY**

THE POWER OF CURATIVE HYPEREMIA

In minor muscular aches and pains due to exertion or fatigue, simple neuralgia and minor muscular strains

In numerous disorders of the joints or muscles, your patient suffers from a vicious cycle of pain causing vasoconstriction, and of vasoconstriction causing pain. To help remedy this condition, apply RUBIGUENT.

RUBIGUENT contains methyl nicotinate, the potent new penetrative agent, and histamine dihydrochloride, a powerful vasodilator. Methyl nicotinate makes it possible for the histamine and the glycol monosalicylate to penetrate tissues, where they promote prolonged, pain-relieving hyperemia with beneficial local warmth.

SUPPLIED: TUBES OF ONE OUNCE

RUBIGUENT*

RUBEFACIENT CREAM, WYETH

Rubefacient... counterirritant... local analgesic

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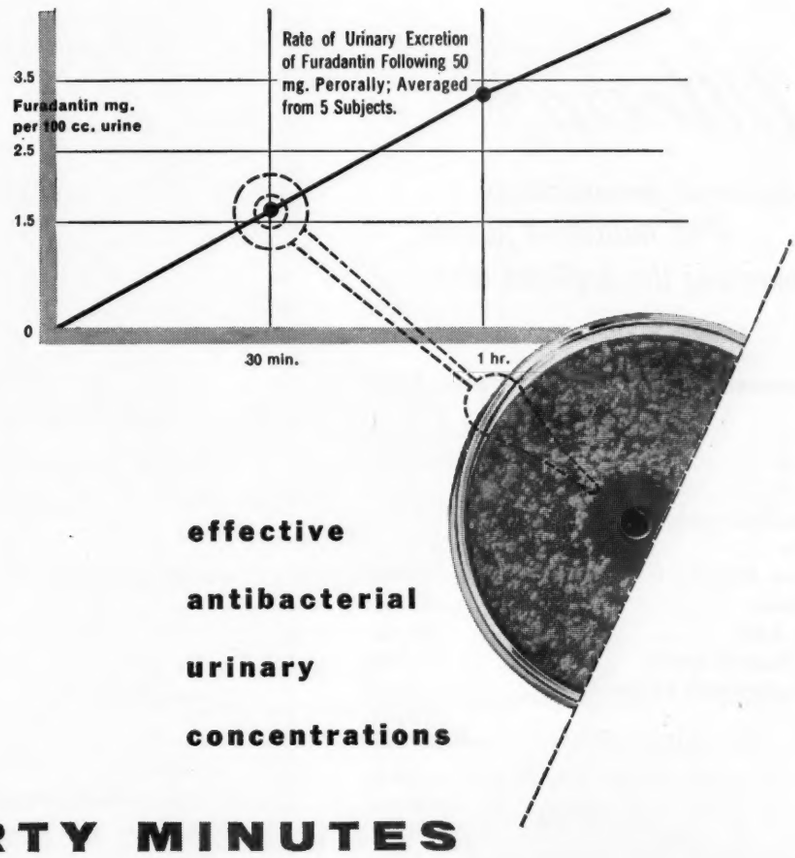

effective antibacterial urinary concentrations

IN THIRTY MINUTES

So remarkable is the affinity of Furadantin for the urinary tract that the urine becomes actively antibacterial within 30 minutes after ingestion, as shown by urinary concentrations and agar plate tests.

Furadantin exhibits an extensive range of antibacterial activity against both gram-positive and gram-negative urinary tract invaders.

Scored tablets of 50 & 100 mg.

IN ACUTE AND CHRONIC URINARY INFECTIONS

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BRAND OF NITROFURANTOIN, EATON

ACCEPTED COUNCIL ON PHARMACY AND CHEMISTRY AMERICAN MEDICAL ASSOCIATION

EATON LABORATORIES Inc.

NORWICH, NEW YORK

THE NITROFURANS—A UNIQUE CLASS OF ANTIMICROBIALS O_2N R **PRODUCTS OF EATON RESEARCH**

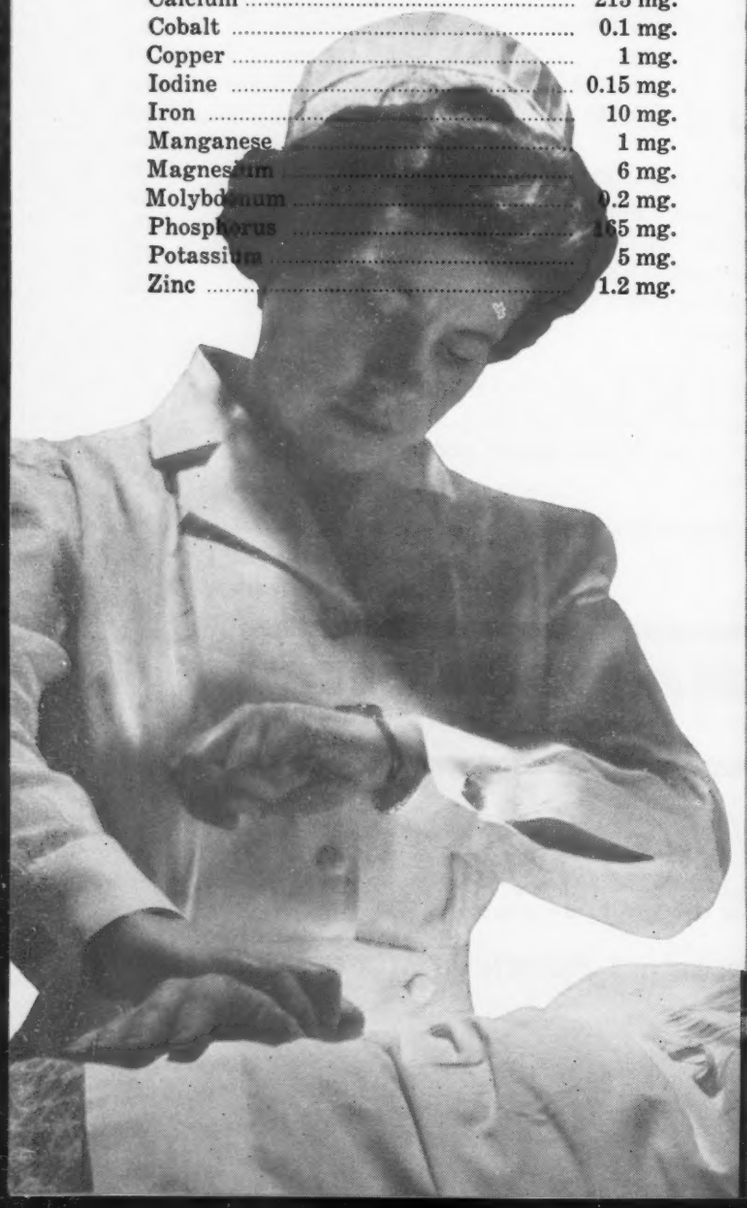
to support the healthy...

Viterra®

a vitamin-mineral formulation of 21 balanced factors, supplementing the depleted diet

each capsule of *Viterra* contains:

Vitamin A	5,000 U.S.P. Units
Vitamin D	500 U.S.P. Units
Vitamin B_{12}	1 mcg.
Thiamine Hydrochloride	3 mg.
Riboflavin	3 mg.
Pyridoxine Hydrochloride	0.5 mg.
Niacinamide	25 mg.
Ascorbic Acid	50 mg
Calcium Pantothenate	5 mg.
Mixed Tocopherols *(Type IV)*	5 mg.
Calcium	213 mg.
Cobalt	0.1 mg.
Copper	1 mg.
Iodine	0.15 mg.
Iron	10 mg.
Manganese	1 mg.
Magnesium	6 mg.
Molybdenum	0.2 mg.
Phosphorus	165 mg.
Potassium	5 mg.
Zinc	1.2 mg.



to fortify the sick...

Viterra® Therapeutic

high-potency capsules specifically designed to meet increased nutritional needs during illness

each capsule of

Viterra Therapeutic

contains:

Vitamin A	25,000 U.S.P. Units
Vitamin D	1,000 U.S.P. Units
Thiamine Mononitrate	10 mg.
Riboflavin	5 mg.
Vitamin B_{12}	5 mcg.
Niacinamide	100 mg.
Ascorbic Acid	150 mg.
Calcium	103 mg.
Cobalt	0.1 mg.
Copper	1 mg.
Iodine	0.15 mg.
Iron	10 mg.
Magnesium	6 mg.
Manganese	1 mg.
Molybdenum	0.2 mg.
Phosphorus	80 mg.
Potassium	5 mg.
Zinc	1.2 mg.

ROERIG Rx

J. B. ROERIG AND COMPANY

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maalox®

gives ulcer relief

without side effects

Gastric hyperacidity is controlled by Maalox-Rorer without constipation or other side effects commonly encountered with antacids. Relief of pain and epigastric distress is prompt and long-lasting. Available in tablets and liquid form.

Suspension Maalox-Rorer contains the hydroxides of Magnesium and Aluminum in colloidal form. The smooth texture and pleasant flavor make it highly acceptable, even with prolonged use.

Supplied: in 355 cc. (12 fluid ounce) bottles. Also in bottles of 100 tablets. (Each Maalox tablet is equivalent to 1 fluidram of Suspension Maalox.)

Samples will be sent promptly on request.

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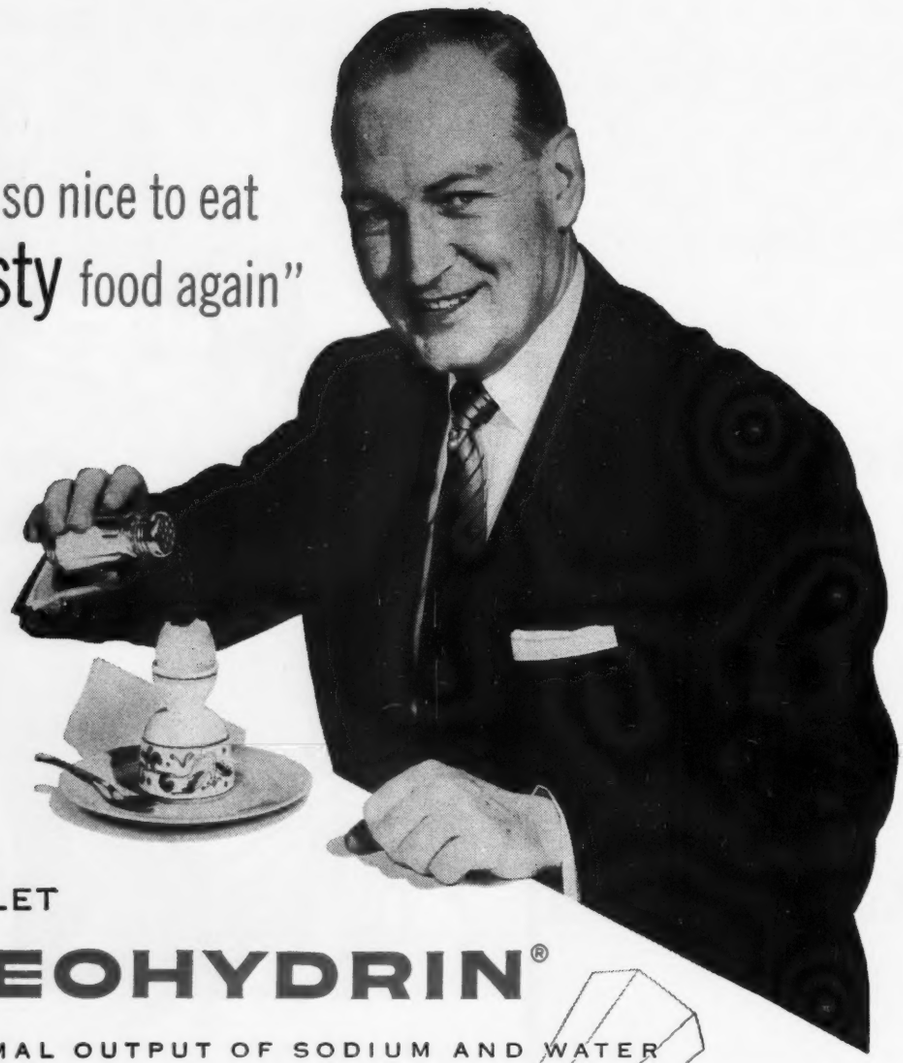
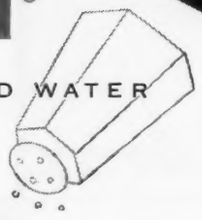


TABLET

NEOHYDRIN®

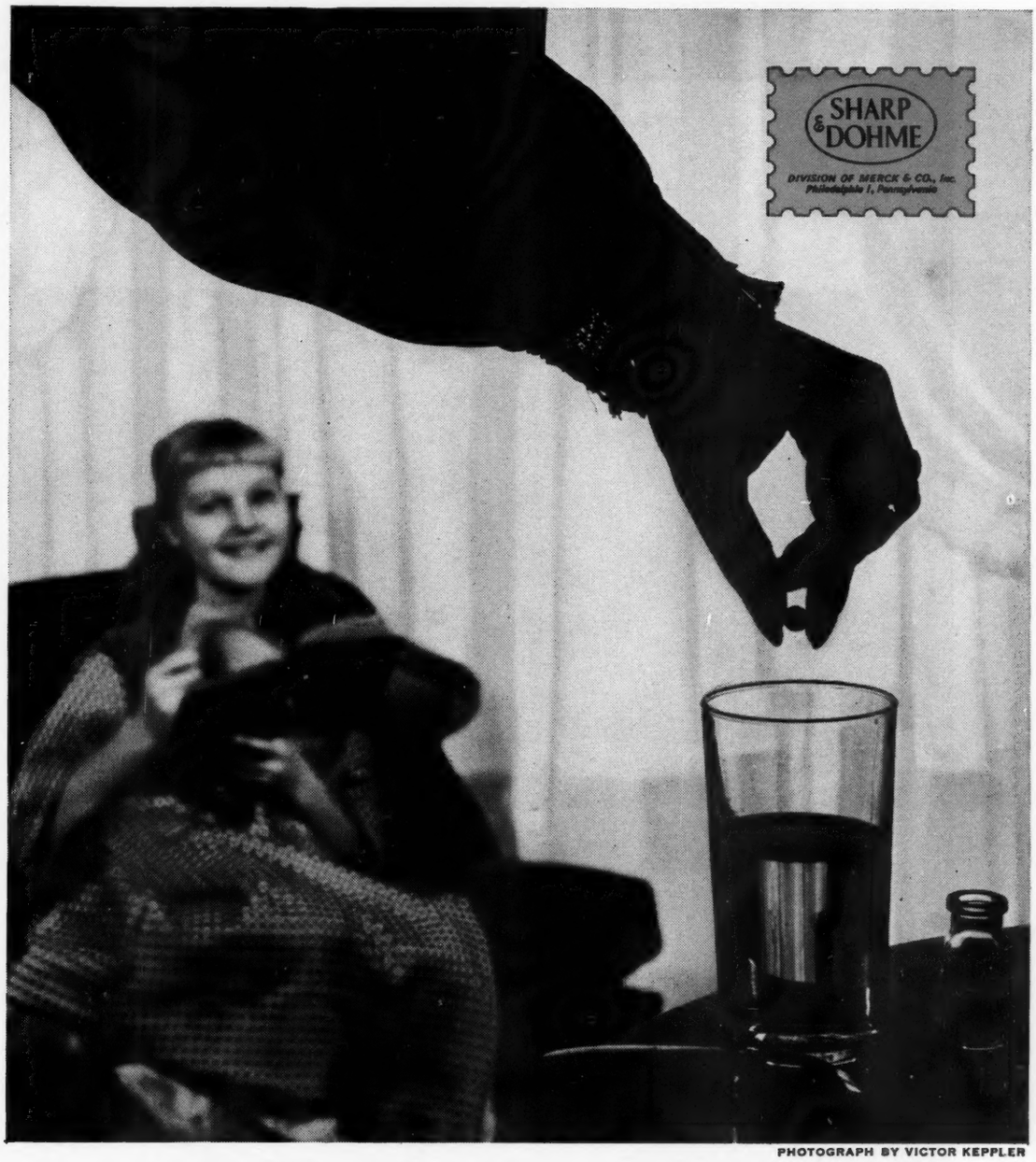
NORMAL OUTPUT OF SODIUM AND WATER



PRESCRIBE NEOHYDRIN whenever there is retention of sodium and water except in acute nephritis and in intractable oliguric states. You can balance the output of salt and water against a more physiologic intake by individualizing dosage. From one to six tablets a day, as needed.

PRESCRIBE NEOHYDRIN in bottles of 50 tablets. There are 18.3 mg. of 3-chloromercuri-2-methoxy-propylurea in each tablet.

Leadership in diuretic research
Lakeside LABORATORIES, INC., MILWAUKEE 1, WISCONSIN



PHOTOGRAPH BY VICTOR KEPPLER

Penicillin—in her favorite beverage

PENALEV®

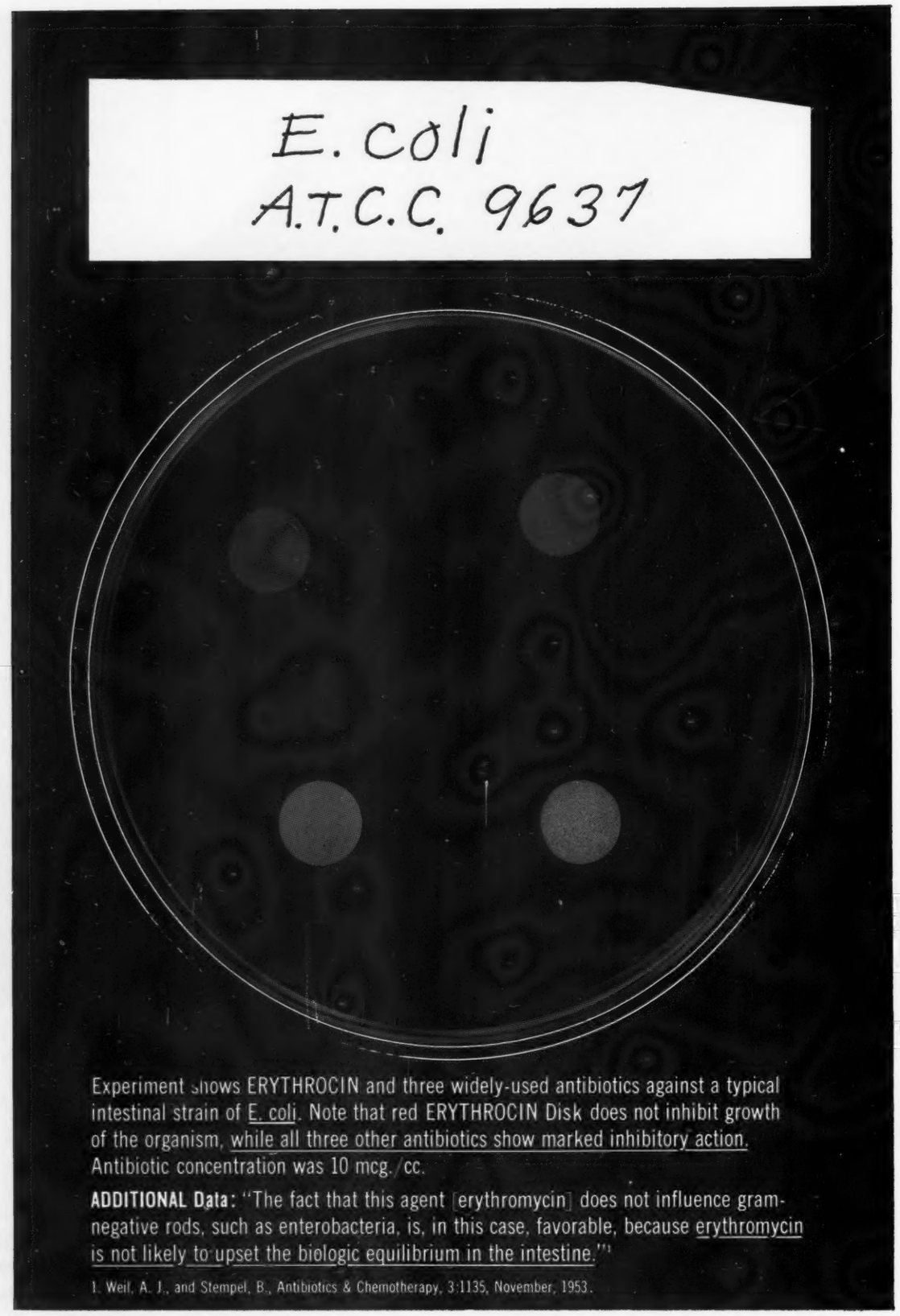
SOLUBLE TABLETS POTASSIUM PENICILLIN G

Readily soluble in milk, fruit juice, infant formulas, PENALEV is a highly agreeable way to give penicillin to your young patients. "The rheumatic child who should receive routine year-round penicillin prophylaxis,"[1] won't object to penicillin when she can drink it.

PENALEV is effective in all infections where oral penicillin is indicated. Its solubility makes it a useful form for regulation of dosage. Convenient for prescription compounding, too.

Quick Information: PENALEV is supplied in soluble tablets of 50,000, 100,000, 200,000, 250,000, 500,000, 1,000,000 units of potassium penicillin G each. Dosage depends on type and severity of the infection.

Reference: 1. J.A.M.A. 151:347, 1953.



Experiment shows ERYTHROCIN and three widely-used antibiotics against a typical intestinal strain of E. coli. Note that red ERYTHROCIN Disk does not inhibit growth of the organism, while all three other antibiotics show marked inhibitory action. Antibiotic concentration was 10 mcg./cc.

ADDITIONAL Data: "The fact that this agent [erythromycin] does not influence gram-negative rods, such as enterobacteria, is, in this case, favorable, because erythromycin is not likely to upset the biologic equilibrium in the intestine."[1]

1. Weil, A. J., and Stempel, B., Antibiotics & Chemotherapy, 3:1135, November, 1953.

Less likely to alter normal intestinal flora

ERYTHROCIN

TRADE MARK

(Erythromycin, Abbott)

the selective antibiotic

NOW, *no problem of gastroenteral disturbances. Unlike many antibiotics,* ERYTHROCIN *is specific in action—spares normal flora, destroys invading cocci. (See the dramatic Sensitivity Test on the opposite page.)*

Highly active, ERYTHROCIN is effective against most coccal infections. It's especially advantageous against staphylococci, because of the high incidence of staphylococci resistant to other antibiotics. Also, prescribe ERYTHROCIN when patients are sensitive to other oral antibiotics.

Average adult dose is 200 mg. every 4 to 6 hours. ERYTHROCIN tablets (100 and 200 mg.) come in bottles of 25 and 100. Abbott

1-94-54

tested

broad-spectrum therapy

Terramycin®

BRAND OF OXYTETRACYCLINE

established

an agent of choice

in the treatment of a wide range of infections due to gram-positive and gram-negative bacteria, spirochetes, rickettsiae, certain large viruses and protozoa.

clinical advantages known

rapid absorption — Within an hour after oral administration in fasting or non-fasting state, effective serum concentrations of Terramycin may be attained.[1] It is widely distributed in body fluids, organs and tissues and diffuses readily through the placental membrane.[2,3]

wide distribution

prompt response — Immediate evidence of Terramycin's efficacy is often obtained by the rapid return of temperature to normal.[4]

excellent toleration — Widely used among patients of all ages, this tested broad-spectrum antibiotic is well tolerated,[5] often when other antibiotics are not.[6]

1. Sayer, R. J., et al.: Am. J. M. Sc. *221*:256 (Mar.) 1951.
2. Welch, H.: Ann. New York Acad. Sc. *53*:253 (Sept.) 1950.
3. Werner, C. A., et al.: Proc. Soc. Exper. Biol. & Med. *74*:261 (June) 1950.
4. Wolman, B., et al.: Brit. M. J. *1*:419 (Feb. 23) 1952.
5. Potterfield, T. G., et al.: J. Philadelphia Gen. Hosp. *2*:6 (Jan.) 1951.
6. King, E. Q., et al.: J. A. M. A. *143*:1 (May 6) 1950.

Available in convenient oral, parenteral and topical forms.

Pfizer

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NOW: for Adults and Children!

Nephenalin®
(for adults)

Nephenalin®
PEDIATRIC

First, under tongue for quick asthma relief

from aludrine (Isopropyl arterenol) HCl in coating

Then, swallow tablet for prolonged asthma control

from theophylline, ephedrine, phenobarbital, contained in tablet core

NEPHENALIN, the "relay-action" tablet combining two widely prescribed, complementary anti-asthmatics, is now available in two potencies: the familiar square, *purple* tablet for adults, and the smaller square, *red* tablet for children. Since a single NEPHENALIN tablet provides quick asthma relief, thereby often replacing the nebulizer, and since relief lasts about four hours, many asthmatic patients will find it the most convenient and efficient anti-asthmatic they have ever used. Bottles of 20 and 100 tablets.

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March, 1954

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for the patient with moderate or severe essential hypertension

THE PATIENT REPORTS
aggressive relief of hypertensive symptoms, if present.

YOU observe
benefits in up to 80% of cases: e.g., hypertension gradually reduced, renal circulation improved, eye-ground changes may be reversed.

THE LITERATURE REPORTS
Therapy is generally well tolerated with initial low dosages gradually increased. Patient response is the guide to dosage adjustment.* Optimal maintenance dosage level is usually reached only after 4, 6 or more weeks; marked therapeutic effect cannot be expected with initial low dosages.*

*References

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